Published in final edited form as:

Ann Thorac Surg. 2013 July ; 96(1): . doi:10.1016/j.athoracsur.2013.04.013.

Interleukin-1 Beta Induces an Inflammatory Phenotype in Human Aortic Valve Interstitial Cells Through Nuclear Factor Kappa Beta

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Abstract

Background—Mechanisms of inflammation have been implicated in the pathogenesis of aortic stenosis. When stimulated, human aortic valve interstitial cells (AVICs) have been shown to become inflammatory cells. Increased levels of interleukin (IL)-1 have been found in the leaflets of stenotic aortic valves. The purpose of this study was to determine the effects of IL-1 on isolated human AVICs and to determine the intracellular signaling pathway by which the effects are mediated. The results of this study demonstrated that IL-1 induces an inflammatory phenotype in human AVICs.

Methods—Human AVICs were isolated from normal aortic valves from explanted hearts of patients undergoing cardiac transplantation (n = 4) and grown in culture. When grown to confluence, the cells were treated with IL-1 (10 ng/mL). Cell culture media was analyzed for IL-6, IL-8, and monocyte chemoattractant protein-1 (enzyme-linked immunosorbent assay). Cell lysates were analyzed for intercellular adhesion molecule-1 (immunoblot). Inhibition of nuclear factor- κ B was by Bay 11-7085 (5 μ M). Inhibition of extracellular signal regulated kinase-1/2 was by PD098059 (20 nM). Statistics were by analysis of variance, with *p* less than 0.05 significant.

Results—Interleukin-1 induced an inflammatory phenotype in human AVICs. The IL-1 stimulation resulted in significantly increased production of the inflammatory cytokines, IL-6 and IL-8, the chemokine monocyte chemoattractant protein-1, and intercellular adhesion molecule-1. Inhibition of nuclear factor- κ B prevented these changes, whereas inhibition of extracellular signal regulated kinase-1/2 had no effect.

Conclusions—Interleukin-1 induced an inflammatory phenotype in human AVICs, which was prevented by inhibition of nuclear factor- κ B. These data implicate IL-1 in the pathogenesis of aortic stenosis.

Calcific aortic stenosis has long been considered a “degenerative” disease, characterized by the passive accumulation of calcium on the aortic valve leaflets. Recently, however, we and others have demonstrated that calcific aortic stenosis may in fact be an active disease process; mechanisms of inflammation and osteogenesis appear to play important roles in the pathogenesis of aortic stenosis [1–6]. As such, aortic stenosis may be an inflammatory disease.

The aortic valve interstitial cell (AVIC) has been implicated in the pathogenesis of aortic stenosis [7, 8]. Under basal conditions, AVICs have a phenotype best described as that of a myofibroblast [6]. In response to proinflammatory stimulation, the phenotype of human AVICs may be transformed into that of an inflammatory cell [9–11]. Characteristics of this inflammatory cell phenotype include production of proinflammatory cytokines and chemokines [11]. Inflammatory changes in human AVICs may also cause the AVICs to assume an osteogenic phenotype [6], characterized by the production of the potent bone-forming protein, bone morphogenetic protein-2, the osteogenic transcription factor, Runx2, and an increased expression and activity of alkaline phosphatase [6]. Hence, there is a linkage between mechanisms of inflammation and osteogenesis in AVICs. A better understanding of this linkage will lead to a better understanding of the pathogenesis of calcific aortic stenosis.

Histologic examination of stenotic aortic valve leaflets has demonstrated increased levels of the proinflammatory cytokine, interleukin (IL)-1 [12]. Interleukin-1 is produced by circulating monocytes, and is among the most potent proinflammatory cytokines studied to date. It has been implicated in the pathogenesis of many inflammatory diseases, including aortic stenosis [12, 13]. The proinflammatory actions of IL-1 are mediated by the activation of intracellular signaling pathways [13]. Although the intracellular signaling pathways activated by IL-1 appear to vary among different cell types, IL-1 has been shown to activate nuclear factor (NF)- κ B in some cells and to activate mitogen-activated protein kinases such as extracellular signal-regulated kinase (ERK)-1/2 in other types of cells [14–18]. This is especially noteworthy because our laboratory has previously demonstrated that intracellular signaling by both NF- κ B and ERK1/2 are present in human AVICs [6, 19].

The finding of increased levels of IL-1 in calcified aortic valve leaflets does suggest that IL-1 may play a role in the pathogenesis of aortic stenosis. This is particularly intriguing because IL-1 has been shown to play a role in many inflammatory diseases. However, to date, no mechanistic role for IL-1 has been identified in the pathogenesis of aortic stenosis. Given that the inflammatory actions of AVICs have been previously implicated in the pathogenesis of aortic stenosis, we hypothesized that the proinflammatory actions of IL-1 stimulate human AVICs to express an inflammatory phenotype.

The objective of this study was to examine the effect of IL-1 on isolated human AVICs from nonstenotic aortic valve leaflets. The first purpose was to examine the effect of IL-1 stimulation on the production of (1) the proinflammatory cytokines IL-6 and IL-8, (2) the chemokine monocyte chemoattractant protein (MCP)-1, and (3) the intercellular adhesion molecule (ICAM)-1. The second purpose was to determine the intracellular signaling pathway by which the effects of IL-1 are mediated in AVICs. We specifically investigated whether its actions were mediated by NF- κ B or by ERK1/2.

Material and Methods

This study was approved by the Colorado Multiple Institutional Review Board of the University of Colorado School of Medicine. All patients provided written informed consent.

Chemicals and Reagents

Medium 199 was purchased from Lonza (Walkersville, MD). The NF- κ B inhibitor Bay 11-7085 was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The ERK1/2 inhibitor, PD098059, was purchased from Calbiochem (San Diego, CA). Rabbit polyclonal antibody against human ICAM-1 was purchased from Prosci (Poway, CA). Antibodies directed against NF- κ B (#8242), phosphorylated NF- κ B (#4806), ERK1/2 (#9102), and phosphorylated ERK1/2 (#9101) were purchased from Cell Signaling Technology (Danvers,

MA). Protein assay reagents and chemiluminescent substrate (ECL) were purchased from Thermo Scientific (Rockford, IL). Four percent to 20% gradient polyacrylamide Ready gels, nitrocellulose membranes, and 2X Laemmli sample buffer were purchased from Bio-Rad (Hercules, CA). The MCP-1, IL-6, and IL-8 ELISA kits were purchased from R&D Systems (Minneapolis, MN). All other chemicals were purchased from Sigma Chemical (St. Louis, MO).

Cell Isolation and Culture

Grossly normal aortic valve leaflets were obtained from the explanted hearts of patients undergoing cardiac transplantation at the University of Colorado Hospital (n = 4 patients). All leaflets were thin, pliable, and grossly normal without any calcification. Cell isolation was by collagenase digestion as previously described, and AVICs were cultured and maintained as independent cultures in medium 199 with penicillin G, streptomycin, amphotericin B, and 10% fetal bovine serum in an incubator supplied with 5% carbon dioxide [6]. The techniques that were used for isolation and culture of AVICs have previously been described in detail [6]. Briefly, aortic valves were treated under sterile conditions in the operating room and placed immediately into 4°C in sterile saline. After three vigorous washes with sterile saline, the valves were sectioned and segments were either placed into 4% formaldehyde in phosphate-buffered saline (PBS), flash frozen, or placed in optimal cutting temperature compound for frozen sections. (These techniques were used to assure that the leaflets are histologically normal as well as grossly normal.) The remaining sections were washed five times with Earl's Balanced Salt Solution placed in 2.5 mg/mL collagenase in full medium 199 for 30 minutes and incubated at 37°C. The supernatant was disposed and valve sections were washed once with Earl's Balanced Salt Solution to remove endothelial cells. Aortic valve segments underwent further digestion for 3 hours in 0.8 mg/mL collagenase in full medium 199 and cells were pelleted by centrifugation, resuspended in full medium 199, and grown in culture (Passage zero). As previously described in detail [6], two microscopic techniques are utilized to assure that the cells grown in culture and studied are AVICs (myofibroblasts) and a homogenous population: light microscopy is used to demonstrate a homogeneous morphology, and immunofluorescent microscopy is used to confirm that cells stain positively for antigens that delineate them as myofibroblasts: vimentin and alpha-smooth muscle actin. Representative images have previously been published [6]. Cells from passages 3 to 6 were used for all experiments grown to 70% to 90% confluence and subcultured to 24-well plates for immunoblotting experiments.

AVIC Stimulation With IL-1 β

The IL-1 β was dissolved in sterile PBS. In a separate experiment, the concentration-response to IL-1 β was studied over a concentration range of 1 ng/mL to 20 ng/mL (data not presented). The maximal response of AVICs to IL-1 β was found to be 10 ng/mL, and this concentration was therefore used in the present study. In another separate experiment, the production of inflammatory mediators was studied as a function of the time of exposure of AVICs to IL-1 β (10 ng/mL) at times from 5 to 90 minutes (data not presented). The response of AVICs to IL-1 β was found to be maximal at 60 minutes. This time was therefore used for the present study.

AVIC NF- κ B Inhibitor Treatments

In the experiments in which NF- κ B was inhibited, AVICs that were treated with NF- κ B inhibition were first pre-treated with the NF- κ B inhibitor, Bay 11-7085 (5 μ M) (dissolved in dimethyl sulfoxide [DMSO] 0.02%) for 30 minutes in serum-free medium, serum-free medium with DMSO 0.02% as a vehicle control, and serum-free medium alone (control). Media were aspirated and IL-1 β 10 ng/mL added to the collected media and then returned to

their respective wells. Cells were washed twice with cold PBS and were lysed using 1X Laemmli sample buffer with 1:38 β -mercaptoethanol and cell scraping.

AVIC ERK1/2 Inhibitor Treatments

In the experiments in which ERK1/2 was inhibited, AVICs that were treated with ERK1/2 inhibition were first pre-treated with the ERK1/2 inhibitor, PD 098059 (20 nM) dissolved in DMSO for 30 minutes in serum-free medium, serum-free medium with DMSO as a vehicle control, and serum-free medium alone (control). Media were aspirated and IL-1 β 10 ng/mL was added to the collected media and then returned to their respective wells. Cells were washed twice with cold PBS and were lysed using 1X Laemmli sample buffer with 1:38 β -mercaptoethanol and cell-scraping.

Immunoblotting

Immunoblotting was used to analyze (1) ICAM-1 production in cell lysates; (2) total NF- κ B and phosphorylated NF- κ B; and (3) total ERK1/2 and phosphorylated ERK1/2. The AVICs in culture were lysed using 1X Laemmli sample buffer with β -mercaptoethanol. Lysates were loaded into 15-well 4% to 20% gradient Ready gels (Bio-Rad) and run at 200 V for 35 minutes. Transfer was to nitrocellulose membranes at 100 V for 70 minutes then cross-linked using a UV Stratalinker (Stratagene, La Jolla, CA) twice, then blocked using 5% dry milk in 0.1% Tween in PBS (T-PBS). After three washes with 0.1% T-PBS, the blocked membranes were incubated overnight at 4°C with primary antibodies that were diluted (1:1000 to 1:10,000) in 5% BSA in 0.1% T-PBS. Again, after three washes in 0.1% T-PBS, membranes were incubated in appropriate horseradish peroxidase-conjugated secondary antibodies diluted to 1:5000 in 5% dry milk in 0.1% T-PBS for 1 hour at room temperature. After three washes in 0.1% T-PBS, membranes were incubated in ECL for 5 minutes at room temperature and exposed on X-ray film. Images were scanned using a flatbed scanner (Epson, Long Beach, CA), and images were analyzed using the National Institutes of Health densitometry software, Image J.

Enzyme-Linked Immunosorbent Assay

Enzyme-linked immunosorbent assays for MCP-1, IL-6, and IL-8 were done using kits purchased from R&D Systems. Cell culture medium, 100 μ L, was used to detect the cytokine levels. The plates were read using a Synergy H1 Hybrid Reader by BioTek (Winooski, VT).

Statistical Analysis

Data are presented as means $\pm$ SE, and statistical analysis was performed using analysis of variance with significance defined as p less than 0.05.

Results

Patient Demographics

All valves were obtained from patients who underwent cardiac transplantation for idiopathic dilated cardiomyopathy (all male, aged 23 to 60 years; $n = 4$ patients). No patients had hypertension, hyperlipidemia, or renal insufficiency. No patients used tobacco or had a history of tobacco usage. All patients underwent coronary angiography and none had any evidence of coronary artery disease. All patients had many echocardiograms before cardiac transplantation, and none had any echocardiographic evidence of aortic stenosis or regurgitation. All patients had trileaflet aortic valves. No patient had dilation or aneurysm formation of the ascending aorta.

IL-1 β Induced Inflammatory Phenotype in Human AVICs

The IL-1 induced a significant increase in the production of the proinflammatory cytokines IL-6 (Fig 1A) and IL-8 (Fig 1B), the chemokine MCP-1 (Fig 1C), and the adhesion molecule ICAM-1 (Fig 1D; $p < 0.05$). For each of these inflammatory mediators, pretreatment with the NF- κ B inhibitor Bay 11-7085 prevented the IL-1-stimulated increase.

IL-1 β Activates NF- κ B in Human AVICs

The data presented in Fig 1 strongly suggested that the effects of IL-1 were mediated by intracellular signaling through NF- κ B. Therefore, we determined whether NF- κ B was activated (phosphorylated) by IL-1. As shown in Figure 2, phosphorylation of NF- κ B was found after 30 minutes of exposure to IL-1 and peaked at 60 minutes. Thereafter, the expression of phospho-NF- κ B promptly declined (data not shown). Note that DMSO, the solvent vehicle used in other experiments in the present study, did not stimulate expression of phospho-NF- κ B.

The NF- κ B Inhibitor Bay 11-7085 Prevents IL-1 β -Induced Activation of NF- κ B

Other investigators have reported that Bay 11-7085 is a specific inhibitor of NF- κ B. However, heretofore, it has not been studied in AVICs. As shown in Figure 3, stimulation of isolated human AVICs with IL-1 (10 mg/mL for 60 minutes) significantly activated NF- κ B. This IL-1-induced activation of NF- κ B was prevented by pretreatment with Bay 11-7085. Note that the activation of NF- κ B by IL-1 alone was not different from that of IL-1 plus DMSO, the solvent vehicle for the NF- κ B inhibitor, Bay 11-7085.

IL-1 β Did Not Activate ERK1/2 in Isolated Human AVICs

Exposure of isolated human AVICs to IL-1 (10 mg/mL) for as long as 60 minutes failed to activate ERK1/2 (Fig 4). There was no difference in phospho-ERK1/2 over baseline control for as long as 60 minutes of stimulation by IL-1.

Inhibition of ERK1/2 Had No Effect on Induction of IL-1 β -Induced Inflammatory Phenotype

As shown in Figure 5, IL-1 induced a significant increase in the production of the proinflammatory cytokines IL-6 (Fig 5A) and IL-8 (Fig 5B), the chemokine MCP-1 (Fig 5C), and the adhesion molecule ICAM-1 (Fig 5D; $p < 0.05$). However, inhibition of ERK1/2 (PD 098059 (20 nM)) had no effect on the actions of IL-1.

Comment

Despite the prevalence of calcific aortic stenosis, the mechanisms responsible for the disease remain unclear. However, some clues to the pathogenesis of the disease have come from histology examination of diseased aortic valve leaflets. Such histologic studies have demonstrated evidence of inflammation and osteogenesis in leaflets of stenotic aortic valves explanted at the time of aortic valve replacement surgery [1–6]. We have previously demonstrated that in response to proinflammatory stimuli, AVICs from normal aortic valve leaflets assume an inflammatory phenotype [11]. We have also previously demonstrated that once the AVIC becomes an inflammatory cell, it can further change to an osteogenic phenotype [6]. Data such as these have implicated the AVIC in the pathogenesis of aortic stenosis; aortic stenosis may derive from inflammatory responses of the AVIC leading to osteogenesis.

Interleukin-1 is one of the most potent proinflammatory cytokines that has been studied to date [13]. It is synthesized in circulating mononuclear cells [13]. When mononuclear cells invade tissues, IL-1 is released and exerts its proinflammatory effects on the surrounding

tissues. These facts are noteworthy because neither IL-1 nor monocytes have been found in normal aortic valve leaflets, but both have been found in stenotic aortic valve leaflets. For this reason, IL-1 has been implicated in the pathogenesis of aortic stenosis. However, heretofore, no study has demonstrated a mechanism by which IL-1 might play a role in the pathogenesis of aortic stenosis.

The results of the present study demonstrate an important mechanism by which the inflammatory actions of human AVICs may be initiated. When stimulated by IL-1, isolated human AVICs assumed an inflammatory phenotype. This inflammatory phenotype was marked by the production of the adhesion molecule ICAM-1, the proinflammatory cytokines IL-6 and IL-8, and the chemokine MCP-1. Further, the results of the present study demonstrate that these actions of IL-1 are mediated through NF- but not ERK1/2.

There are limitations to the present study that must be acknowledged. First, isolated AVICs were studied in vitro. As with any study of isolated cells, it is a limitation that the behavior of the cells in vitro may change as the cells are grown in cell culture. However, we have previously demonstrated that isolated human AVICs that have been grown through multiple passages in cell culture have functions comparable to those of freshly isolated cells [6]. A second limitation of any study of isolated cells is that it is not possible to understand whether the behavior of cells studied in vitro may differ from those in vivo. For example, it is not possible to know how cell-cell interactions in vivo may affect the responses seen in vitro. A third limitation is the relatively small sample size. However, the responses to IL-1 of the valves from each of the 4 human subjects were qualitatively and quantitatively very consistent; statistical significance was easily reached. It therefore seems very unlikely that results with a larger sample size would differ. Finally, in the present study, AVICs from normal aortic valve leaflets rather than stenotic valves were studied. The actions of IL-1 on AVICs from stenotic leaflets may therefore differ from the findings of the present study. However, the purpose of the present study was to determine whether IL-1 may induce the types of changes in human AVICs that have been suggested to lead to the development of aortic stenosis. It was therefore appropriate to study AVICs from normal aortic valve leaflets.

Despite these limitations, the findings of the present study have important implications. To our knowledge, the present study is the first to demonstrate a mechanism by which IL-1 may play an important role in the pathogenesis of aortic stenosis.

Interleukin-1 is recognized to play a role in the pathogenesis of many inflammatory diseases, including rheumatoid arthritis, nephritis, and colitis [13–15]. After mononuclear cells infiltrate into tissues, IL-1 is released and it then exerts its inflammatory actions. Given the histologic evidence that inflammation plays an important role in aortic stenosis combined with the finding of IL-1 in diseased aortic valve leaflets, the proinflammatory actions of IL-1 have been implicated in the pathogenesis of aortic stenosis. Histologic examination of stenotic aortic valve leaflets by Kaden and colleagues [12] revealed the presence of IL-1 in mononuclear cell infiltrates within the leaflets; conversely, leaflets from normal valves did not demonstrate IL-1. These investigators also demonstrated that IL-1 promotes matrix metalloproteinase expression in aortic valve leaflets [12]. The results of the present study have now demonstrated that IL-1 may incite an inflammatory cascade by inducing an inflammatory phenotype in the human AVIC. Hence, the findings of the present study implicate IL-1 in the pathogenesis of aortic stenosis.

The AVIC has been implicated in the pathogenesis of calcific aortic stenosis [6, 7]. Work from our laboratory has previously shown that when stimulated by mechanisms of inflammation acting through Toll-like receptor 4, AVICs demonstrate increased expression

of proinflammatory genes and proteins [11]. In turn, we have also previously shown that such an inflammatory response is linked to mechanisms of osteogenesis [6, 9, 10]; mechanisms of inflammation have been demonstrated to incite mechanisms of osteogenesis in human AVICs. The results of the present study imply that IL-1 may play a role in initiating such an inflammatory response in AVICs.

The proinflammatory actions of IL-1 are initiated at the cellular level when IL-1 binds to the interleukin-1 membrane-bound receptor (ILR). Once the IL-1 membrane-bound receptor is stimulated, it in turn has the ability to activate intracellular signaling pathways, including those mediated through NF- κ B and ERK1/2. Work by other investigators has demonstrated that the particular intracellular signaling pathway that is activated by IL-1 may be tissue-specific [16–20]. In prior studies, we have previously demonstrated that both NF- κ B and ERK1/2 intracellular signaling pathways may be activated in human AVICs [6, 21] as a function of a specific stimulating agonist. The results of the present study demonstrate that in human AVICs, NF- κ B, but not ERK1/2 was activated by IL-1. Because we did not demonstrate IL-1-induced activation of ERK1/2, it is not surprising that the actions of IL-1 were not prevented by inhibition of ERK1/2. It was important, however, to demonstrate that the data were internally consistent. Conversely, the IL-1-induced production of inflammatory mediators was prevented by inhibition of NF- κ B. In the present study, NF- κ B inhibition was accomplished with Bay 11-7085, a standard pharmacologic experimental tool used for this purpose. Its mechanism of action is through an irreversible inhibition of cytokine-induced phosphorylation of I κ B-alpha, which in turn, results in an inhibition of NF- κ B [22, 23]. The results of the present study suggest, therefore, that the intracellular signaling induced by IL-1 is mediated by NF- κ B.

Work from our laboratory has previously reported a deficiency in calcified aortic valve leaflets of the specific antiinflammatory mechanism responsible for blocking the actions of IL-1, IL-1 receptor antagonist [15]. Such a deficiency leaves the proinflammatory actions of IL-1 unchecked, and therefore even more potent. The results of the present study demonstrate for the first time the powerful proinflammatory actions of IL-1 on isolated human AVICs; IL-1 was shown to induce an inflammatory phenotype in isolated AVICs from normal aortic valve leaflets. In concert with the results of prior work [13–15], the results of the present study suggest the possibility that an imbalance of the proinflammatory actions of IL-1 and its antiinflammatory defense mechanism, IL-1 receptor antagonist, in vivo may play an important role in the pathogenesis of calcific aortic stenosis.

In summary, the results of the present study demonstrate that IL-1 induces an inflammatory phenotype in AVICs isolated from normal aortic valve leaflets. Characteristics of this inflammatory phenotype include the production of proinflammatory cytokines, the chemokine MCP-1, and the adhesion molecule ICAM-1. The results of the present study also offer insight into the intracellular mechanisms of action of IL-1. In the present study, IL-1 activated NF- κ B, and inhibition of NF- κ B blocked the production of IL-1-induced inflammatory mediators. Therefore, the results of the present study provide evidence that links a powerful inflammatory cytokine, IL-1, to the pathogenesis of aortic stenosis and provides insight into its intracellular mechanisms.

Acknowledgments

Funded by grants from the American Heart Association (11GRNT7900016) and the National Institutes of Health (RO1 HL106582-01).

Abbreviations and Acronyms

AVIC	aortic valve interstitial cell
ERK	extracellular signal-regulated kinase
ICAM	intercellular adhesion molecule
IL	interleukin
MCP	monocyte chemoattractant protein
NF	nuclear factor
PBS	phosphate-buffered saline
T-PBS	Tween in phosphate-buffered saline

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Fig 1.

Interleukin (IL)-1 induced an inflammatory phenotype in isolated human aortic valve interstitial cells (AVICs). After stimulation with IL-1, the expression of proinflammatory cytokines (A) IL-6 and (B) IL-8, (C) the chemokine monocyte chemoattractant protein (MCP)-1 and (D) intercellular adhesion molecule (ICAM)-1 were significantly increased (* $p < 0.05$). Treatment with the nuclear factor- κ B inhibitor Bay 11-7085 prevented these responses. The IL-6, IL-8, and MCP-1 levels were measured by enzyme-linked immunosorbent assay, and ICAM-1 by Western blot and densitometry. Dimethyl sulfoxide (DMSO) is a vehicle control.

Fig 2.

Stimulation with interleukin (IL)-1 induced phosphorylation of nuclear factor (NF)- κ B. Human aortic valve interstitial cells (AVICs) were treated with IL-1 (10 ng/mL). Western blots using cell lysate were done for phosphorylated (phospho) and total NF- κ B. There was some activation of NF- κ B at 5 minutes. The activation was increased at 30 minutes and was maximal at 60 minutes. (DMSO = dimethyl sulfoxide.)

Fig 3.

Stimulation with interleukin (IL)-1 induced activation of nuclear factor (NF)- κ B that was prevented by Bay 11-7085. Human aortic valve interstitial cells (AVICs) were treated for 60 minutes with either IL-1 (10 ng/mL), IL-1 (10 ng/mL) plus dimethyl sulfoxide (DMSO) 0.02%, or IL-1 (10 ng/mL) plus Bay 11-7085 (5 μ M). Western blots using cell lysates demonstrate phosphorylated (phospho) and total NF- κ B. DMSO (0.02%) was the solvent-vehicle for Bay 11-7085 and had no effect on phospho-NF- κ B level.

Fig 4. Stimulation with interleukin (IL)-1 did not activate extracellular signal regulated kinase (ERK)1/2. Human aortic valve interstitial cells (AVICs) were treated with IL-1 (10 ng/mL). Western blots using cell lysate were done for phosphorylated (phospho) and total ERK1/2. (DMSO = dimethyl sulfoxide.)

Fig 5. Stimulation with interleukin (IL)-1 (10 ng/mL) induced significantly increased expression of (A) the cytokines IL-6 and (B) IL-8, (C) the chemokine monocyte chemoattractant protein (MCP)-1, and (D) the intercellular adhesion molecule (ICAM)-1 in human aortic valve interstitial cells (AVICs). This expression is not affected by addition of the extracellular-signal regulated kinase (ERK)1/2 inhibitor PD098059. (* $p < 0.05$). (DMSO = dimethyl sulfoxide.)