Cross-Talk Between the Toll-Like Receptor 4 and Notch1 Pathways Augments the Inflammatory Response in the Interstitial Cells of Stenotic Human Aortic Valves

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Background and Purpose—Calcific aortic stenosis is a chronic inflammatory disease, and aortic valve interstitial cells (AVIC) play an important role in valvular inflammation. Whereas AVIC from stenotic aortic valves exhibit an augmented response to Toll-like receptor 4 (TLR4) stimulation, the underlying mechanism is unclear. This study tested the hypothesis that an excessive cross-talk between the TLR4 and Notch1 pathways is responsible for augmentation of the inflammatory response to lipopolysaccharide (LPS) in AVIC of stenotic valves.

Methods and Results—Human AVIC were isolated from normal and stenotic leaflets. Nuclear factor kappa-B (NF-κB) activation and production of interleukin-8, monocyte chemoattractant protein-1, and intercellular adhesion molecule-1 were analyzed after treatment with LPS. The role of Notch1 in the inflammatory response was determined using inhibitor, siRNA, and specific ligand. Cells from diseased valves produced greater levels of chemokines and intercellular adhesion molecule-1 that are associated with enhanced NF-κB activation. Interestingly, diseased cells exhibited augmented Jagged1 release and Notch1 activation after TLR4 stimulation. Inhibition and silencing of Notch1 each resulted in greater suppression of the TLR4-induced inflammatory response in diseased cells. Conversely, activation of Notch1 with a specific ligand, Jagged1, enhanced the LPS-induced inflammatory response in normal AVIC. Further, Notch1 intracellular domain was communoprecipitated with the inhibitor of NF-κB kinase after LPS stimulation, and inhibition of Notch1 abrogated the difference in the level of NF-κB activation between diseased and normal cells.

Conclusion—Notch1 enhances the inflammatory response to TLR4 stimulation in human AVIC through modulating NF-κB activation. Excessive cross-talk between the TLR4 and Notch1 pathways is responsible for augmentation of the TLR4 response in AVIC of stenotic valves. (Circulation. 2012;126[suppl 1]:S222–S230.)

Key Words: inflammation ■ Notch1 ■ signal transduction ■ Toll-like receptor 4 ■ valves

Calcific aortic stenosis is a leading cardiovascular disease in older people and is recognized as a chronic inflammatory disease. With the increase in the aging population, there is a surge in the incidence of this cardiovascular disease. However, the mechanisms responsible for the development of calcific aortic stenosis remain incompletely understood. Pharmacological interventions for prevention of aortic valve calcification and its progression to calcific stenosis rely on a thorough understanding of the mechanisms. Explanted human aortic valve leaflets exhibit evidence of inflammation. Chronic periodontal infection may play a role in the pathogenesis of calcific aortic stenosis. In this regard, oral bacteria have been found in stenotic aortic valves, and inoculation of rabbits with oral bacteria induces aortic valve lesions. Endothelial cells on aortic valve surface interact with aortic valve interstitial cells (AVIC) to maintain the integrity of valve tissues. Studies indicate that abnormal hemodynamic forces (such as elevated pressure and shear stresses) experienced by the valve leaflets can cause endothelial injury that could lead to valve inflammation and tissue remodeling. It is possible that endothelial injury or dysfunction is an early event of the disease process of calcific aortic stenosis. However, because inflammation and calcification occur within the valve tissue, AVIC play an important role in the pathogenesis of calcific aortic stenosis. In this regard, AVIC have been found to express osteogenic proteins in response to proinflammatory cytokine stimulation. We found that human AVIC express functional Toll-like receptor 4 (TLR4), an important signaling receptor in the innate immunity.
immune response and inflammation. Stimulation of TLR4 with lipopolysaccharide (LPS) in human AVIC induces the inflammatory and osteogenic responses.9,10 Examining the mechanism of TLR4-induced inflammatory response in human AVIC of stenotic valves may provide insights into the pathogenesis of calcific aortic stenosis.

Our previous study found that AVIC of stenotic valves express higher levels of bone morphogenetic protein-2, an inflammasomegenic mediator, in response to TLR4 stimulation with LPS.10 However, the mechanism underlying the enhanced response to TLR4 stimulation in AVIC of diseased valves remains unclear. Bacterial lipopeptide and LPS have been found to induce Notch1 activation in macrophages.11 Notch proteins (Notch1–4) are transmembrane receptors expressed on the cell surface. On ligand binding, Notch receptors undergo proteolytic (Notch1–4) are transmembrane receptors expressed on the cell surface. On ligand binding, Notch receptors undergo proteolytic

necrosis factor-α (NF-κB), the master switch for proinflammatory gene expression.14 Notch1 is an important modulator of cellular inflammatory response and contributes to the mechanism underlying the enhanced response of AVIC of stenotic valves to TLR4 stimulation with LPS. Key questions are whether AVIC of diseased valves have exaggerated Notch1 activation in response to TLR4 stimulation and, if they do, how Notch1 modulates the inflammatory response in AVIC.

The purpose of this study is to determine: (1) whether Notch1 activation in response to TLR4 stimulation is enhanced in human AVIC of stenotic valves; (2) whether Notch1 plays a role in augmentation of the inflammatory response to TLR4 stimulation in diseased AVIC; and (3) whether Notch1 modulates TLR4-mediated activation of nuclear factor kappa-B (NF-κB), the master switch for proinflammatory gene expression.

Materials and Methods

Antibody against intercellular adhesion molecule (ICAM)-1, Notch1 siRNA, and scrambled siRNA were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Antibodies against Notch1, NICD1, phosphorylated NF-κB p65, and total NF-κB p65 were purchased from Cell Signaling (Beverly, MA). Medium 199 was purchased from Lonza (Walkersville, MD). Recombinant Jagged1 and cytokine–enzyme-linked immunosorbent assay kits were purchased from R&D System (Minneapolis, MN). Jagged1 enzyme–linked immunosorbent assay kit was purchased from Uscn Life Science. LPS (Escherichia coli 0111:B4) and all other chemicals and reagents were purchased from Sigma-Aldrich Chemical (St Louis, MO).

Cell Isolation and Culture

Normal aortic valve leaflets were collected from the explanted hearts of 6 patients (4 males and 2 females; mean age, 59.9 ± 8.1 years) undergoing heart transplantation and stenotic valves were obtained from 8 patients (5 males and 3 females; mean age, 66 ± 11.3 years) undergoing aortic valve replacement. All patients gave informed consent for the use of their valves for this study. This study was approved by the Colorado Multiple Institutional Review Board of the University of Colorado Denver.

AVIC were isolated and cultured using a previously described method with modifications.8,14 Briefly, valve leaflets were subjected to sequential digestions with collagenase, and cells were collected by centrifugation. Cells were cultured in M199 growth medium containing penicillin G, streptomycin, amphotericin B, and 10% fetal bovine serum. When the cells reached 80% to 90% confluence, they were subcultured on plates and chamber slides for the experiments. Cells from passages 4 to 6 were used for this study.

Cells were stimulated with LPS (200 ng/mL) for 8 to 24 hours to measure levels of proinflammatory mediators (IL-8, monocyte chemoattractant protein [MCP]-1, and ICAM-1), Notch1 activation, and Jagged1 release. Cells were stimulated with LPS for 1 hour to 8 hours to assess NF-κB activation (NF-κB p65 phosphorylation and intranuclear translocation).

To determine the role of Notch1 in the inflammatory response, cells were treated with DAPT (50 μM) or Notch1 siRNA (60 nmol/L) before stimulation with LPS. To determine the effect of Notch1 activation on the inflammatory response, cells were cultured on plates coated with Jagged1, a specific Notch1 ligand, and stimulated with LPS.

Immunoblotting

Immunoblotting was applied to analyze Notch1, NICD1, ICAM-1, phosphorylated NF-κB p65, and total NF-κB p65. Cells were lysed in a sample buffer (100 mmol/L Tris- HCl, pH 6.8, 2% SDS, 0.02% bromophenol blue, and 10% glycerol).

Protein samples were separated on gradient (4%–20%) mini-gels and transferred onto nitrocellulose membranes (Bio-Rad Laboratories, Hercules, CA). The membranes were blocked with 5% nonfat dry milk solution for 1 hour at room temperature. The blocked membranes were incubated with primary antibody against Notch1, NICD1, ICAM-1, phosphorylated NF-κB p65, or total NF-κB p65. After washing with phosphate-buffered saline containing 0.05% Tween 20, the membranes were incubated with a peroxidase-linked secondary antibody specific to the primary antibody. After further washes, membranes were treated with enhanced chemiluminescence reagents. Then the membrane was exposed on x-ray film. Image J was used to measure the density of bands.

Enzyme-Linked Immunosorbent Assay

Cell culture supernatants were collected and levels of IL-8, MCP-1, and Jagged1 were determined using enzyme-linked immunosorbent assay kits as described previously.15

Immunofluorescent Staining

Immunofluorescent staining was applied to localize NF-κB p65 as described previously.14 After permeabilization with a methanol/acetone mixture, cells on chamber slides were fixed in 4% paraformaldehyde and incubated with the primary antibody (mouse monoclonal antibody against human NF-κB p65) overnight at 4°C. After washing with phosphate-buffered saline, cells were incubated with Cy3-tagged secondary antibody against the primary antibody (imaged on the red channel). Nuclei were stained with bis-benzimide (DAPI, imaged on the blue channel) and glycoproteins on cell surfaces with Alexa 488-tagged wheat germ agglutinin (imaged on the green channel). Microscopy was performed with a Leica DMRXA digital microscope (Leica Mikroskopie und Systeme GmbH, Wetzlar, Germany) equipped with Slidebook software (I L I., Denver, CO).

Gene Knockdown

Notch1 silencing was performed using the method described previously.16 Human AVIC were cultured in antibiotic-free growth medium until 60% confluent. The cells were incubated with a mixture of siRNA specific to human Notch1 (60 nmol/L) and transfection reagent (6 μL per ml medium) in antibiotic-free and serum-free medium for 6 hours. After transfection, cells were incubated in growth medium for 48 hours and then stimulated with LPS. Control cells were treated with scrambled siRNA (sc-37007) and transfection reagent (sc-29526).

Communoprecipitation

Cells were lysed in TNT solution (50 mmol/L Tris-HCl, 200 mmol/L NaCl, and 1% Triton X-100; pH 7.5), and the lysates were centrifuged at 735g for 10 minutes at 4°C. Supernatants were preclarea
by incubation with 25 μL of 1:1 slurry of Gamma Bind-Sepharose (Amersham Pharmacia) for 2 to 3 hours at 4°C on a rocking platform. After centrifugation at 14,000 g rpm for 30 seconds, cleared lysates were incubated with a rabbit polyclonal antibody to human inhibitor of NF-κB kinase-β (IKK-β) (2.0 μg/sample) overnight at 4°C with rocking. Fifty μL of the 1:1 Gamma Bind-Sepharose slurry was added to each sample, and samples were incubated at 4°C for additional 4 to 6 hours. Immune complexes, collected by centrifugation at 16,000 g for 3 seconds, were washed in ice-cold TNT solution and ice-cold phosphate-buffered saline, and solubilized by the addition of 25 μL of SDS sample buffer (100 mmol/L Tris-HCl, 2% SDS, 0.02% bromophenol blue, and 10% glycerol; pH 6.8). Each sample was subjected to SDS-polyacrylamide gel electrophoresis, and IKK-β and NICD1 were detected with monoclonal antibodies.

**Statistical Analysis**

Data are presented as mean ± SE. Statistical analysis was performed using StatView software (Abacus Concepts, Calabasas, CA). ANOVA with the post hoc Bonferroni/Dunn test and t test were used to analyze differences between experimental groups, and differences were confirmed with Mann–Whitney U tests. For data with a time course, 2-way ANOVA was used to compare the difference between experimental groups at each time point. An interaction was also tested if a linear trend was indicated. Statistical significance was defined as \( P \leq 0.05 \).

**Results**

**AVIC of Stenotic Valves Exhibit a Greater Inflammatory Response to TLR4 Stimulation**

Figure 1A and 1B show that the release of IL-8 and MCP-1 and expression of ICAM-1 were significantly higher in AVIC of stenotic valves than those in AVIC of normal valves after stimulation for 24 hours with TLR4 agonist LPS. A, The levels of interleukin (IL)-8 and monocyte chemoattractant protein (MCP)-1 in the culture medium of cells from stenotic valves are higher than those of cells from normal valves (n=6). B, Representative immunoblots and densitometric data (n=4) show that cells from stenotic valves exhibit higher intercellular adhesion molecule-1 (ICAM-1) protein levels after LPS stimulation than cells from normal valves. C, Representative immunoblots and densitometric data (n=4) show that cells from stenotic valves exhibit enhanced NF-κB phosphorylation after treatment with LPS for 1 to 8 hours. D, Representative images show robust and sustained intranuclear localization of NF-κB p65 in AVIC of stenotic valves. *\( P \leq 0.05 \) vs corresponding control value. †\( P \leq 0.05 \) vs normal cells receiving the same treatment.
group and the stenotic group was significant ($P=0.016$). Similarly, AVIC of stenotic valves exhibited augmented NF-$\kappa$B p65 intranuclear translocation (Figure 1D). In addition, intranuclear localization of NF-$\kappa$B p65 lasted longer in AVIC of stenotic valves (Figure 1D). Therefore, the augmented inflammatory response to TLR4 stimulation in AVIC of stenotic valves is associated with enhanced NF-$\kappa$B activation.

AVIC of Stenotic Valves Have Exaggerated Notch1 Activation After TLR4 Stimulation

As shown in Figure 2A, TLR4 stimulation induces Notch1 activation in AVIC of normal valves and stenotic valves. NICD1 was detectable at 4 hours with TLR4 stimulation, and NICD1 accumulation was evident with prolonged TLR4 stimulation. Interestingly, markedly higher levels of NICD1 were observed in AVIC of stenotic valves (Figure 1D). TLR4 stimulation also caused the release of Jagged1 in AVIC of both normal and stenotic valves. Jagged1 levels in culture media increased at 4 hours after exposing cells to LPS and remained elevated at 24 hours (Figure 2B). The release of Jagged1 was significantly enhanced in AVIC of stenotic valves (Figure 2B).

Inhibition and Silencing of Notch1 Attenuates the Inflammatory Response to TLR4 Stimulation

To determine the role of the enhanced Notch1 activation in the augmented inflammatory response to LPS in AVIC of stenotic valves, we applied DAPT, a $\gamma$-secretase inhibitor, to inhibit the generation of NICD1. We pretreated AVIC of normal valves and stenotic valves with DAPT for 1 hour and then stimulated cells with LPS. Treatment with DAPT essentially abrogated the generation of NICD1 at 8 hours and greatly reduced NICD1 levels at 24 hours of TLR4 stimulation in cells from both normal and diseased valves (Figure 3A). Importantly, chemokine release and ICAM-1 expression were markedly reduced in cells treated with DAPT, and a greater reduction was observed in AVIC of stenotic valves (Figure 3B, C). Similarly, Notch1 knockdown reduced chemokine production (not shown) and ICAM-1 expression after TLR4 stimulation (Figure 4). These results demonstrate that Notch1 signaling plays an important role in mediating the TLR4-induced inflammatory response in AVIC, and that enhanced Notch1 activation is responsible, at least in part, for the enhanced inflammatory response in AVIC of stenotic human valves.

Activation of Notch1 Enhances the Inflammatory Response to TLR4 Stimulation

To further determine the effect of Notch1 activation on the inflammatory response to TLR4 stimulation, we cultured normal cells on Jagged1-coated plates and stimulated them with LPS. Figure 5 show that Jagged1 markedly enhanced the inflammatory response to LPS in AVIC from normal valves, although Jagged1 alone had a minor effect on cytokine production. These results provide a further support to our hypothesis that Notch1 signaling contributes to the mechanism of the augmented inflammatory response to TLR4 stimulation in AVIC of stenotic valves.

Notch1 Modulates NF-$\kappa$B Activation in Human AVIC

To determine whether Notch1 links TLR4 to NF-$\kappa$B activation in human AVIC, we treated cells of stenotic aortic valves with DAPT and normal AVIC with Jagged1, followed by LPS stimulation for 1, 2, 4, or 8 hours. NF-$\kappa$B p65 phosphorylation was analyzed in the individually treated samples. Treatment with DAPT markedly reduced NF-$\kappa$B p65 phosphorylation in diseased cells and essentially abolished the difference in the level of NF-$\kappa$B p65 phosphorylation be-
tween normal and diseased cells (Figure 6A). Conversely, treatment of normal cells with Jagged1, a specific Notch1 ligand, enhanced TLR4-induced NF-κB p65 phosphorylation (Figure 6B). Further, NICD1 was coimmunoprecipitated with IKK-α after TLR4 stimulation (Figure 6C). Together, these results demonstrate that Notch1 signaling links TLR4 to NF-κB activation, and that the enhanced NF-κB response in diseased AVIC is primarily attributable to enhanced Notch1 activation in response to TLR4 stimulation. It appears that Notch1 interacts with IKK to modulate NF-κB activation.

Discussion

Accumulating evidence indicates that inflammation plays an important role in the pathogenesis of calcific aortic stenosis.17 We previously reported that human AVIC exhibit an inflammatory response to TLR4 stimulation with LPS and that the inflammatory response is augmented in cells of stenotic aortic valves.9,10 The results of the present study show that enhanced Notch1 activation in response to TLR4 stimulation mediates the augmented inflammatory response in AVIC of stenotic valves. The Notch1 pathway augments the inflam-
matory response through modulation of NF-κB activation (summarized in Figure 7). The present study uncovered important differences in the inflammatory response between AVIC from normal versus diseased valves, and highlighted the importance of the cross-talk between the TLR4 and Notch1 signaling pathways in AVIC of stenotic valves.

The Enhanced Inflammatory Response in AVIC of Stenotic Aortic Valves Is Mediated by Excessive Cross-Talk Between TLR4 and Notch1

Chronic periodontal infection and bacterial wall components have been shown to play a role in the pathogenesis of vascular calcification and atherosclerosis. Several studies demonstrate that TLR2 knockout and TLR4 knockout attenuate vascular atherosclerotic injury. Explanted human aortic valve leaflets exhibit evidence of inflammation. Further, bacteria associated with chronic periodontal infection have been detected in stenotic aortic valves, and inoculation of rabbits with oral bacteria induces aortic valve lesions. However, the role of TLR in aortic valve stenosis remains elusive. We recently observed that stimulation of TLR4 with LPS upregulates bone morphogenetic protein-2 expression in human AVIC. Our finding suggests a link between innate immune receptors and the osteogenic response in human AVIC and supports the hypothesis that proinflammatory signaling in AVIC has a mechanistic role in the development and progression of calcific aortic stenosis.

In this study, we found that greater production of IL-8, MCP-1, and ICAM-1, in response to TLR4 stimulation, in AVIC of stenotic valves correlates with enhanced NF-κB activation, expressed as phosphorylation and intranuclear translocation. Because these proinflammatory mediators are important for leukocyte infiltration, the inflammatory response of AVIC may be involved in initiation and exacerbation of valvular tissue inflammation in the disease process. The finding that AVIC of stenotic valves have an augmented

![Figure 4](http://circ.ahajournals.org/)

**Figure 4.** Knockdown of Notch1 attenuates intercellular adhesion molecule-1 (ICAM-1) expression after Toll-like receptor 4 (TLR4) stimulation with lipopolysaccharide (LPS) in cells from stenotic valves. Aortic valve interstitial cells (AVIC) of stenotic aortic valves are treated with Notch1 siRNA and then stimulated with LPS. A, Representative immunoblots of 3 experiments show that treatment with Notch1 siRNA reduces Notch1 intracellular domain (NICD1) levels after LPS stimulation. B, Representative immunoblots of 3 experiments show that knockdown of Notch1 reduces ICAM-1 expression in AVIC of stenotic valves after TLR4 stimulation.

![Figure 5](http://circ.ahajournals.org/)

**Figure 5.** Activation of Notch1 enhances lipopolysaccharide (LPS)-induced inflammatory response in normal aortic valve interstitial cells (AVIC). A, Jagged1 enhances LPS-induced chemokine production in cells from normal valves (n=4). B, Representative immunoblots and densitometric data (n=3) show that treatment with Jagged1 enhances intercellular adhesion molecule-1 (ICAM-1) expression in cells from normal valves after LPS stimulation. *P<0.05 vs control; †P<0.05 vs LPS alone.
inflammatory response to TLR4 stimulation suggests that AVIC of stenotic valves have a proinflammatory phenotype.

The Notch signaling pathway regulates cell differentiation, proliferation, survival, and development. In addition, Notch signaling modulates cytokine production in T lymphocytes and macrophages. However, it is unknown whether Notch1 is involved in augmentation of the inflammatory response to TLR4 stimulation in AVIC of diseased valves.

We found that AVIC of normal valves and stenotic valves exhibit Notch1 activation after TLR4 stimulation in a time-dependent manner, and Notch1 activation is more prominent in AVIC of diseased valves. Further, AVIC releases Notch1 ligand Jagged1 after TLR4 stimulation with LPS, and AVIC of diseased valves release markedly higher levels of Jagged1. Thus, the elevated production of proinflammatory mediators in AVIC of stenotic valves is associated with enhanced Notch1 activation. It appears that AVIC of diseased valves have an enhanced TLR4–Notch1 cross-talk. Although the signaling pathway that triggers Jagged1 release in human AVIC remains to be identified, elevated Jagged1 release appears to be responsible for enhanced Notch1 activation in diseased AVIC.

**Notch1 Plays an Important Role in Augmentation of the TLR4-Induced Inflammatory Response in AVIC of Diseased Valves**

To define the role of Notch1 activation in the inflammatory response to TLR4 stimulation, we determined the effects of inhibition of Notch1 cleavage with DAPTabolished the difference in NF-κB phosphorylation between cells from normal and diseased valves. In addition, Notch1 knockdown markedly attenuated the inflammatory response in diseased cells. Interestingly, the effect of Notch1 inhibition on the inflammatory response is more profound in AVIC of diseased valves.
Notch1 activity plays an important role in augmentation of the inflammatory response in AVIC of stenotic valves.

To further define the role of Notch1 in modulation of the inflammatory response in human AVIC, we stimulated cells from normal valves with LPS in the presence of specific Notch1 ligand Jagged1. Interestingly, activation of Notch1 with Jagged1 augmented TLR4-induced inflammatory response. This finding highlights the importance of Notch1 interaction with TLR4 signaling in mediating the inflammatory response in AVIC and provides further evidence in support of the notion that Notch1 augments the inflammatory response to TLR4 stimulation with LPS in human AVIC.

It has been reported that stimulation with LPS upregulates the expression of Notch1 and its ligand Jagged1 in murine BV-2 cells.²⁵ The results of the present study show that AVIC of stenotic valves release more Jagged1 and exhibit enhanced Notch1 activation after TLR4 stimulation. It is likely that the increased Jagged1 release in AVIC of stenotic valves mediates the enhanced Notch1 activation and the augmented inflammatory response to TLR4 stimulation. It is noteworthy that we and others found higher levels of TLR4 protein in AVIC of stenotic valves.¹⁰,²⁶ However, the density of TLR4 on the cell surface is comparable in AVIC from normal valves and stenotic valves.²⁶ Further, the results of the present study show that inhibition of Notch1 abrogated the differences in inflammatory mediator production between diseased and normal cells, indicating a major role for enhanced Notch1 activation in augmentation of the inflammatory response to TLR4 stimulation in diseased AVIC.

A limitation of this study is the focus only on AVIC. Endothelial cells may be involved in aortic valve inflammation. However, AVIC may have a major role in the overall inflammatory response of aortic valve tissue because AVIC are the main cell type in aortic valve and inflammation occurs within the valve tissue.⁷ Thus, it is likely that Notch1 modulates the overall inflammatory response of aortic valve tissue through its impact on AVIC. Further studies are needed to determine the role of Notch1 in the inflammatory response of aortic valve endothelial cells to TLR4 stimulation.

**Notch1 Augments the Inflammatory Response Through Modulation of NF-κB Activation**

A previous report found an effect of Notch1 signaling on NF-κB and protein kinase B activation in macrophages treated with LPS.²⁷ Our results show that Notch1 signaling has a profound effect on NF-κB phosphorylation in human AVIC and that Notch1–NF-κB interaction is enhanced in AVIC of stenotic valves. DAPT, which inhibits Notch1 activation, attenuates NF-κB phosphorylation and abrogates the difference in the level of NF-κB phosphorylation between AVIC of normal and stenotic valves after TLR4 stimulation. Further, activation of Notch1 with Jagged1 enhances NF-κB phosphorylation after TLR4 stimulation. The mechanism underlying the effect of Notch1 on NF-κB phosphorylation appears to involve molecular interaction of NICD1 with IKK because NICD1 is coimmunoprecipitated with IKK after TLR4 stimulation.

**Conclusion**

In conclusion, the present study demonstrates that the cross-talk of TLR4 with Notch1 enhances the inflammatory response to TLR4 stimulation with LPS in human AVIC via modulating NF-κB activity. Enhanced TLR4 interaction with Notch1 is responsible for the augmentation of the inflammatory response to TLR4 stimulation in AVIC of stenotic human valves. The results of the present study provide mechanistic insights into the inflammatory mechanism of calcific aortic stenosis and may lead to identification of therapeutic targets for suppression of valvular inflammation. Modulation of proinflammatory signaling may have a therapeutic potential for treatment of early aortic stenosis.

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Disclosures
None.

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