Genetic and Pharmacologic Disruption of Interleukin-1β Signaling Inhibits Experimental Aortic Aneurysm Formation

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Objective—Abdominal aortic aneurysms (AAAs) are common, but their exact pathogenesis remains unknown and no specific medical therapies are available. We sought to evaluate interleukin-1β (IL-1β) and interleukin-1 receptor (IL-1R) in an experimental AAA model to identify novel therapeutic targets for AAA treatment.

Methods and Results—IL-1β mRNA and protein levels were significantly elevated in abdominal aortas of 8- to 12-week-old male C57BL/6 mice after elastase aortic perfusion (wild-type [WT]) compared with saline perfusion. Mice with genetic deletion of IL-1β (IL-1β knockout [KO]) or IL-1R (IL-1R KO) that underwent elastase perfusion demonstrated significant protection against AAA formation, with maximal aortic dilations of 38.0±5.5% for IL-1β KO and 52.5±4.6% for IL-1R KO compared with 89.4±4.0% for WT mice (P<0.005). Correspondingly, IL-1β KO and IL-1R KO aortas had reduced macrophage and neutrophil staining with greater elastin preservation compared with WT. In WT mice pretreated with escalating doses of the IL-1R antagonist anakinra, there was a dose-dependent decrease in maximal aortic dilation (R²=–0.676; P<0.0005). Increasing anakinra doses correlated with decreasing macrophage staining and elastin fragmentation. Lastly, WT mice treated with anakinra 3 or 7 days after AAA initiation with elastase demonstrated significant protection against AAA progression and had decreased aortic dilation compared with control mice.

Conclusion—IL-1β is critical for AAA initiation and progression, and IL-1β neutralization through genetic deletion or receptor antagonism attenuates experimental AAA formation. Disrupting IL-1β signaling offers a novel pathway for AAA treatment. (Arterioscler Thromb Vasc Biol. 2013;33:1-11.)

Key Words: aneurysm • animal model surgery • aortic disease • interleukin-1 receptor antagonist • vascular inflammation

Abdominal aortic aneurysms (AAAs) affect nearly 7% of older men and account for over 15,000 deaths annually in the United States.1–4 AAA-related mortality is mostly because of aortic rupture, which carries a 75% to 90% mortality rate and is directly related to aortic diameter.4 With no specific medical therapies for AAAs, current strategy involves watchful waiting during AAA progression until the risk of rupture exceeds the risk of surgery, at which time patients may undergo surgical intervention. Therefore, any pharmacological option that could resolve aortic dilation or slow the natural rate of AAA expansion would be a meaningful improvement for patients.7

Inflammation has been implicated as a critical factor in AAA development.8 Aneurysm morphology is characterized by mononuclear cell infiltration, increased proinflammatory cytokines and proteases, elastin breakdown, decreased vascular smooth muscle cells, and increased collagen turnover, all of which are classic inflammatory responses.9–11 Interleukin-1 (IL-1) is a primary effector of the inflammatory cascade and functions as the gatekeeper for inflammation.12 IL-1 consists of 2 distinct proteins (IL-1α and IL-1β) that bind to the same receptor (interleukin-1 receptor [IL-1R]) to stimulate a downstream proinflammatory pathway. Compared with healthy aortas, IL-1β protein is elevated 4-fold in human AAAs, and gene expression is increased by over 10-fold.10–13,14 Furthermore, circulating IL-1β is increased by nearly 10-fold in patients with AAAs.15

Despite the critical role of inflammation in AAA formation and the dramatic upregulation of IL-1β in patients with AAAs, the specific contribution of IL-1β to inflammation during AAA formation remains unknown. We hypothesized that IL-1β would be upregulated in experimental AAA pathogenesis and that neutralization of IL-1β, either through genetic deletion or receptor antagonism, would inhibit AA progression. Accordingly, the purpose of this study was to evaluate the role of IL-1β pathway in experimental AAA pathogenesis, with
the ultimate goal of identifying novel therapeutic targets for the treatment of AAAs.

Materials and Methods

Defining IL-1β in Human AAA Samples

Human AAA tissue was obtained from patients undergoing open AAA repair, and control tissue was obtained from organ transplantation donors. Collection of human tissue was approved by the University of Virginia Institutional Review Board for Health Sciences Research (IRB 13178). Sections from human AAAs and healthy human control aortas were stained with IL-1β antibody (1:100 dilution, Santa Cruz Biotechnology, Santa Cruz, CA) for immunohistochemistry.

Defining IL-1β Levels in the Murine Elastase Aneurysm Model

All animal experimental protocols were approved by the University of Virginia Institutional Animal Care and Use Committee (protocol #3634). Two groups of 8- to 12-week-old wild-type (WT) C57BL/6J mice (Jackson Laboratory, Bar Harbor, ME) underwent abdominal aortic perfusion with either 0.44 U/mL elastase or saline perfusion (WT elastase, WT saline; procedure detailed in online-only Data Supplement). Infrarenal abdominal aortas from both groups were harvested for tissue analysis at days 0, 3, 7, and 14 (n=5 per group per time point). Tissues were analyzed with PCR, enzyme-linked immunosorbent assay (ELISA), and histological staining for IL-1β to evaluate changes in IL-1β throughout the murine elastase AAA model (detailed in the online-only Data Supplement). Studies were performed in triplicate. Murine AAA samples were also evaluated with confocal immunohistochemistry using immunofluorescent staining for IL-1β (1:100 dilution), macrophages with mac-2 antibody (1:1000; Cedarlane, Burlington, ON, Canada), smooth muscle cells with smooth muscle α-actin (SMA; 1:1000; Sigma-Aldrich, St. Louis, MO), and nuclei with DAPI (1:10 000; Invitrogen).

Evaluating Genetic Deletion of IL-1β and IL-1R on AAA Formation

Eight- to twelve-week-old male mice with genetic deletion of IL-1β on a C57Bl/6 background (IL-1β knockout [KO]; n=24) underwent elastase perfusion as above. Aortas were harvested at days 3 and 7 for gelatin zymography (n=5 each), as well as at day 14 (n=14). Aortic dilation was measured with video micrometry on all mice harvested. Mice with genetic deletion of IL-1R on a C57Bl/6 background (IL-1R KO; n=13) were evaluated as a second method to target the IL-1β signaling pathway. Age-matched IL-1R KO mice underwent elastase perfusion, and aortic dilation was measured at the time of harvest. For controls, 8- to 12-week-old male C57Bl/6 mice were perfused with elastase (WT elastase; n=11) or saline (WT saline; n=6) for positive and negative controls, respectively. Aortas from IL-1β KO, IL-1R KO, WT elastase, and WT saline mice were collected for mRNA/protein isolation and histology. Absence of IL-1β and IL-1R were verified via PCR. From the isolated protein, cytokine analysis (R&D systems) of all 4 groups was performed (detailed in the online-only Data Supplement). Aortas at day 14 were examined with immunohistochemistry for macrophages, neutrophils, and elastin. Histology samples were graded by a blinded reviewer using the following scale:

- Macrophage staining: 1=none to few, 2=mild, 3=moderate, 4=extensive
- Neutrophil staining: 1=none to few, 2=mild, 3=moderate, 4=extensive
- Elastin staining: 1=concentric heavy rings of elastin, 2=mild elastolysis, 3=moderate elastolysis, 4=extensive elastolysis

Pretreatment With IL-1R Antagonist

Four groups of WT mice were treated with escalating doses of anakinra (Kineret, Biovitrum, Stockholm, Sweden), a commercially available recombinant IL-1R antagonist, prior to elastase exposure to determine the effect of pharmacological IL-1R antagonism on AAA progression. Anakinra is a competitive antagonist for IL-1R with a half-life of 4 to 6 hours and does not have any significant effects on heart rate or blood pressure. Given the short half-life, continuous release osmotic pumps (Durect, Cupertino, CA) were used for anakinra delivery. Prefilled syringes with 100 mg anakinra/0.67 mL were diluted in saline to concentrations that would deliver 10 mg/kg per day, 30 mg/kg per day, and 100 mg/kg per day anakinra solution to the mice. The control group had osmotic pumps filled with saline only as vehicle. Dosages were chosen based on published literature. Thus, 4 groups of WT mice had pumps loaded with vehicle alone, 10 mg/kg per day, 30 mg/kg per day, or 100 mg/kg per day anakinra solution (n=7 per group). Osmotic pumps were placed subcutaneously in the back, with inhaled isoflurane as anesthesia. Three days later, elastase perfusion was performed. Because of the amount of anakinra required, osmotic pumps were exchanged 6 days after elastase perfusion under inhalational anesthesia. Maximal aortic dilation was measured after 14 days, and aortas were harvested for histology (n=3) or frozen for molecular analysis (n=4) with ELISA for IL-1β.

Treatment With IL-1R Antagonist After AAA Induction

The effects of anakinra treatment were examined at 2 time points after elastase perfusion in WT mice, after AAA initiation: (1) elastase perfusion at day 0 followed by anakinra or vehicle treatment starting on day 3 with aortic harvest on day 14 (n=7 per group) and (2) elastase perfusion at day 0 followed by anakinra or vehicle treatment starting on day 7 with aortic harvest on day 21 (n=5 per group). Anakinra was given at 100 mg/kg per day via osmotic pumps placed subcutaneously, as described above, with saline vehicle used as the control. Because of the amount anakinra used, all pumps were exchanged 7 days after placement. At the time of harvest, maximal aortic dilation was measured with video micrometry. Immunohistochemistry was performed for IL-1β, macrophages, neutrophils, and elastin fibers.

IL-1R KO and WT Bone Marrow Transplantation

At 6 weeks of age, WT and IL-1R KO mice underwent lethal irradiation with 2 separate exposures of 650 Gy radiation 4 hours apart. Bone marrow was extracted from the femur and tibia of 2 WT and 2 IL-1R KO mice. WT and IL-1R KO bone marrow cells (5x10⁶) were injected into tail veins of the irradiated mice to make 4 groups of mice: WT bone marrow into WT recipient mice, WT bone marrow into IL-1R KO recipient mice, IL-1R KO bone marrow cells into WT recipient mice, and IL-1R KO bone marrow cells into IL-1R KO recipient mice (n=15 each). Depletion of host bone marrow by irradiation without bone marrow reconstitution was lethal in IL-1R KO and WT mice. The mice were allowed 4 weeks for bone marrow reconstitution. At 10 weeks, all mice underwent elastase perfusion with aortic harvest at 12 weeks. Maximal aortic dilation was measured, and elastin degradation was analyzed by Verhoeff-Van Gieson stain and graded by a blinded reviewer.

Statistical Methods

Statistical analysis was performed using GraphPad Prism 5 software (GraphPad Software, La Jolla, CA). Maximal aortic dilation (%) was calculated as (maximal aortic diameter–internal control diameter)/maximal aortic diameter×100%, with aortic diameters measured from the same photo while the mouse was alive. The internal control was a small segment of normal abdominal aorta just distal to the renal arteries that was above the proximal ligation and was not perfused with elastase. All measurements were repeated by a blinded reviewer and highly correlated with the initial measurements (Figure I in the online-only Data Supplement). Aortic dilation between groups was compared with 1-way ANOVA with post hoc Tukey corrections applied to determine the significance of individual comparisons with α=0.05. When only 2 groups were compared, the Student t test was used.
for significance. Similar analysis was performed based on maximal aortic diameter alone (Figure II in the online-only Data Supplement). Pearson correlation coefficients (R) were used to determine strength of linear dependence and are reported as R value (95% confidence interval [CI]). Differences between groups for histological grading for macrophage, neutrophil, and elastin staining were tested with the χ² test. Values reported are mean±standard deviation.

Results

Increased IL-1β in Human AAA Tissue

Consistent with previous studies that have demonstrated increased IL-1β protein and gene expression in human AAA tissue, human aortic tissue from AAAs demonstrated increased IL-1β staining (Figure III in the online-only Data Supplement).

IL-1β Is Upregulated in Experimental Elastase AAA Model

Aortic dilation in WT mice significantly increased after elastase exposure compared with perfusion with saline (control). At day 3, no difference in aortic dilation between groups was evident. However, aortic dilation was apparent at day 7 (elastase: 61.8±4.0% versus saline: 47.6±4.1%; P<0.05) and most apparent 14 days after perfusion (elastase: 90.1±3.7% versus saline: 45.8±5.1%; P<0.0001; Figure IV in the online-only Data Supplement).

Uninjured murine aortas had low levels of IL-1β gene expression (Figure 1A). However, 3 and 7 days after elastase perfusion, IL-1β gene expression increased significantly compared with saline perfused aortas, with peak expression at day 3 (Figure 1A). IL-1α expression also increased significantly 3 days after elastase exposure (Figure V in the online-only Data Supplement). IL-1β protein was elevated 3 days after elastase exposure and remained significantly elevated at 7 and 14 days (Figure 1B). By immunohistochemistry, elastase perfused aortic cross sections demonstrated greater IL-1β staining that was mostly confined to the medial and adventitial layers (Figure 1C).

Confocal microscopy demonstrated that IL-1β colocalized within both intramural smooth muscle cells and macrophages in aortas 3 days after elastase exposure (Figure 1D). Aortic samples from days 7 and 14 were also evaluated; however, smooth muscle staining was diminished secondary to decreased smooth muscle marker expression, consistent with previous studies.

IL-1β Signaling Is Critical in AAA Formation

With IL-1β shown to be highly upregulated in both human and experimental AAAs, we tested the hypothesis that IL-1β...
is required for AAA formation by performing elastase perfusion on IL-1β KO mice. IL-1β KO mice had >50% absolute reduction in maximal aortic dilation compared with WT mice at 14 days (IL-1β KO: 38.0±20.4% versus WT elastase: 89.4±13.1%; P<0.005; Figure 3A). Importantly, aortic dilation in IL-1β KO mice was similar to WT mice perfused with saline only (IL-1β KO: 38.0±20.4% versus WT saline: 34.6±17.3%; P=NS; Figure 2A).

As a second method of targeting IL-1β signaling, IL-1R KO mice underwent elastase perfusion. IL-1R KO aortic dilation was similar to IL-1β KO at 14 days after elastase exposure and showed significant protection from AAA formation compared with WT elastase (IL-1R KO: 52.5±16.4% versus WT elastase: 89.4±13.1%; P<0.005; Figure 2A and 2B). No significant differences in aortic dilation were present between IL-1β KO elastase, IL-1R KO elastase, and WT saline groups at 14 days after perfusion. Histologically, there was evidence of significant protection from inflammation in the IL-1β and IL-1R KO animals. WT elastase AAA samples demonstrated moderate-to-extensive macrophage and neutrophil staining of the media and adventitia, whereas both IL-1β KO and IL-1R KO aortas had very little macrophage or neutrophil staining in the aortas (Figure 2C; P<0.0001 for both). Elastin fibers were extensively fragmented in the WT elastase group, but were prominent and preserved in both IL-1β KO and IL-1R KO animals (Figure 2C; P<0.0001).

Given the extensive changes in elastolysis, protease levels were investigated with gelatin zymography to evaluate matrix metalloproteinase (MMP) 2 and 9 levels in IL-1β KO aortas compared with WT aortas at 3 and 7 days after elastase perfusion. Three days after elastase exposure, there was a trend toward decreased active MMP9 in the IL-1β KO mice (P=0.12; Figure 3A). Seven days after elastase perfusion, there was a significant decrease in active MMP9 in the IL-1β KO aortas, when compared with WT elastase aortas (P<0.05). MMP2 levels were not significantly decreased in IL-1β KO mice at days 3 or 7 compared with WT aortas.

To determine other cytokines involved in AAA formation that may be affected by the IL-1β pathway, cytokine array analyses were performed on isolated protein from aortas harvested on day 14 from WT elastase, WT saline, IL-1β KO, and IL-1R KO groups. Aortas from IL-1β KO mice had significantly less IL-1α, regulated and normal T-cell expressed and secreted/chemokine (C–C motif) ligand 5, interleukin-6, and complement component 5a compared with WT elastase controls (P<0.05 for all; Figure 3B). IL-1R KO aortas had decreased IL-1α, regulated and normal T-cell expressed and secreted, and complement component 5a compared with WT elastase aortas.

Figure 2. A, Maximal percentage of aortic dilation is shown for elastase perfused wild-type (WT) mice, saline perfused WT mice, elastase perfused interleukin-1β knockout (IL-1β KO) mice, and elastase perfused interleukin-1 receptor knockout (IL-1R KO) mice, and elastase perfused interleukin-1 receptor knockout (IL-1R KO) mice with representative images displayed (B). *P<0.0005 vs WT elastase. C, Immunohistochemistry from WT elastase, IL-1β KO, and IL-1R KO aortas evaluating macrophages shown in brown, neutrophils shown in brown, and elastin fibers (arrows) shown in black with Verhoeff-Van Gieson stain. Scale bar=200 μm. On the right, histograms are shown indicating the distribution of blinded histological grading for each group (n=6/group). Significant differences existed for macrophage infiltration, neutrophil infiltration, and elastin degradation (P<0.0001 for all by χ² test).
Pharmacological IL-1R Antagonism Prevents AAA Formation

Because we had determined that genetic deletion of IL-1β signaling abrogated AAA formation, we hypothesized that pharmacological inhibition of IL-1β signaling using anakinra would also result in prevention of AAAs. To determine if anakinra could affect AAA formation, osmotic continuous release pumps were placed subcutaneously 3 days before elastase exposure with increasing doses of anakinra solution (Figure 4A). Increasing doses of anakinra resulted in a stepwise attenuation in aortic dilation compared with increasing doses of anakinra solution. Anakinra-treated mice also showed a dose-dependent decrease in macrophage and neutrophil staining, as well as an increase in concentric elastin structure compared with vehicle-treated mice (P<0.005; Figure 4B). Anakinra-treated mice also showed a dose-dependent decrease in aortic IL-1β protein via ELISA (R<0.544 [95% CI, –0.785 to –0.158]; P<0.01; Figure 4C). IL-1β protein levels significantly correlated with aortic dilation (R=0.575 [95% CI, 0.203–0.802]; P<0.01). In summary, selective inhibition of IL-1β signaling via IL-1R antagonism with anakinra decreased AAA formation, consistent with our genetic KO studies.

Anakinra-treated mice also showed a dose-dependent decrease in macrophage and neutrophil staining, as well as an increase in concentric elastin structure compared with vehicle-treated controls (P<0.0001 for all; Figure 4D). Similar to the WT elastase mice in Figure 2, WT mice treated with vehicle demonstrated extensive macrophage staining, moderate-to-extensive neutrophil staining, and extensive elastin...
fragmentation. Mice receiving 30 mg/kg per day and 100 mg/kg per day anakinra had trivial macrophage and neutrophil staining and highly preserved elastin architecture.

Continuous infusion of anakinra through subcutaneously placed osmotic pumps did not have any apparent detrimental side effects on the mice. Weight gain among mice treated with anakinra was not different than in mice treated with vehicle alone, during the 14-day study period (1.45±0.91 g for vehicle, 1.28±0.93 g for 10 mg/kg per day, 2.28±0.93 g for 30 mg/kg per day, and 1.51±1.27 g for 100 mg/kg per day). Of the 28 mice, there were 2 mortalities, 1 mouse treated with vehicle alone as a result of aortic rupture and 1 mouse treated with vehicle alone from over anesthesia during osmotic pump change.

**Treatment of Mice With Anakinra Inhibited AAA Progression**

The preceding results showed pretreatment of mice with anakinra before elastase perfusion inhibited aneurysm formation. To determine if treatment with anakinra could inhibit aneurysm progression once an AAA is initiated or a small AAA exists, mice were treated with anakinra at a dose of 100 mg/kg per day or vehicle begun at either 3 days or 7 days after elastase perfusion with aortic harvest at 14 days.

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**Figure 4.** **A**, Study design to evaluate the effect of anakinra for prevention of abdominal aortic aneurysm (AAA) formation. Subcutaneous osmotic pumps were filled with escalating doses of anakinra solution 3 days before elastase infusion. Because of the amount of drug required, pumps were changed 9 days after insertion. Aortic harvest was performed on day 14. **B**, Maximal aortic diameter with the 4 doses of anakinra. *P=0.005 vs vehicle; †P<0.0005 vs vehicle; and ‡P<0.005 vs 10 mg/kg per day. **C**, Aortic interleukin-1β (IL-1β) protein levels by ELISA for aortas exposed to increasing doses of anakinra. *P<0.05. **D**, Representative histology sections for macrophages, neutrophils, and elastin fibers shown for anakinra at 0, 10, 30, and 100 mg/kg and day. Scale bar=200 μm. Histology slides were graded by a blinded reviewer with grading shown on the right with histograms (n=4/group; P<0.0001 for all by χ² test).
days or 21 days, respectively (Figure 5A). Anakinra-treated mice demonstrated significantly decreased aortic dilation compared with vehicle-treated mice. Anakinra treatment starting on day 3 decreased aortic dilation from 86.4±9.1% (vehicle) to 43.7±22.0% on day 14, \(P<0.0005\) (Figure 5B). When anakinra 100 mg/kg per day was started 7 days after elastase perfusion, maximal aortic dilation at day 21 was 52.9±3.2% compared with 82.4±15.3% with vehicle alone, \(P<0.005\) (Figure 5C). Anakinra treatment reduced intramural IL-1\(\beta\) in the aortic wall by immunohistochemistry (Figure 5D). WT mice treated with anakinra 3 and 7 days after elastase exposure demonstrated significant reductions in macrophage and neutrophil staining (\(P<0.0001\) for both) and reduced elastolysis compared with treatment with vehicle alone (\(P<0.0001\); Figure 5D). These results indicate that the IL-1\(\beta\) pathway is critical not only in the initiation of experimental AAAs, but is also required for aneurysm progression, suggesting that disruption of IL-1\(\beta\) signaling may function as a treatment strategy for AAAs.

Nonbone Marrow-Derived IL-1R Is Required for AAA Development

To elucidate the cell types responsible for the protective effects of IL-1R antagonism, bone marrow transplantation between WT and IL-1R KO mice was performed. We hypothesized that transplantation of IL-1R KO bone marrow would protect against AAA formation. To evaluate the relative contribution of bone marrow-derived IL-1R, 4 groups of chimeric mice were created (Figure 6A): WT bone marrow into WT host mice (WT→WT), IL-1R KO bone marrow into WT host mice (IL-1R KO→WT), WT bone marrow into IL-1R KO host mice (WT→IL-1R KO), and IL-1R KO bone marrow into IL-1R KO host mice (IL-1R KO→IL-1R KO). After bone marrow reconstitution, mice underwent elastase perfusion. Results showed IL-1R KO host mice had significantly decreased aortic dilation, but transplantation of IL-1R KO bone marrow into WT mice did not confer any protection (Figure 6B). As expected, IL-1R KO mice receiving IL-1R KO bone marrow cells were protected from AAA (49.2±6.7% aortic dilation compared...
with 77.4±19.3% for WT→WT). However, transplanting IL-1R KO bone marrow into WT mice was not protective of AAA and resulted in mean aortic dilation of 81.1±15.7%, similar to WT mice that received WT bone marrow. IL-1R KO host mice receiving WT bone marrow had decreased aortic dilation (50.3±15.5%). IL-1R KO host mice receiving either WT or IL-1R KO bone marrow had significantly less elastin degradation by Verhoeff-Van Gieson staining than the WT host mice (Figure 6C). These results suggest that IL-1R in nonmyeloid-derived cells, such as vascular smooth muscle cells, may contribute more to AAA development than IL-1R from bone marrow-derived cells, such as mononuclear cells. Of note, IL-1R KO host mice had a high-lethality after irradiation and bone marrow transplantation with 47% lethality for WT bone marrow into IL-1R KO mice and 73% lethality for IL-1R KO bone marrow into IL-1R KO mice.

**Discussion**

IL-1β upregulation seen in human AAA is similarly present during experimental AAA formation. We showed that IL-1β and IL-1R are critical in experimental AAA formation and offer effective pharmacological targets for AAA prevention and treatment. Genetic disruption of IL-1β signaling through either IL-1β or IL-1R deletion significantly attenuated AAA formation and reduced vascular inflammation. The beneficial effects of this pathway for AAA prevention were likewise seen with pharmacological IL-1R antagonism using anakinra. Together, these data indicate that IL-1β signaling is required for AAA initiation. Importantly, delayed administration of IL-1R antagonist also inhibited aortic dilation and AAA progression, indicating that the IL-1β pathway is required for the progression of small established AAAs. Therefore, IL-1β signaling is required for both AAA formation and progression, and IL-1β neutralization has great potential as an AAA treatment strategy.

There is a tremendous need for medical therapy to treat the multitude of patients with AAAs. The current treatment paradigm is observation followed by surgical intervention; importantly, no specific medical options exist to reduce or slow AAA expansion. Based on AAA expansion rates from the UK Small Aneurysm trial, the period of close observation between diagnosis and surgery frequently lasts more than 5 years. During this time of AAA progression, there is a 0.5% to 5% annual risk of aortic rupture. Therefore, this period of watchful-waiting offers great opportunity for medical therapy to reduce AAA size or slow AAA expansion. For example, if rate of expansion of a 3.5-cm AAA could be reduced by just 40%, the need for surgical intervention would be delayed by more than 3 years.

We have shown that IL-1β is critical for both the initiation and progression of experimental AAAs. The most significant increase in IL-1β gene expression and protein occurred early in the AAA formation before any apparent aortic dilation. The drastic increase in IL-1β early in AAA formation suggests that IL-1β upregulation may be one of the key inciting events leading to aneurysm formation. IL-1β is known to lead the inflammatory cascade and trigger expression of many proinflammatory cytokine genes, including the cyclic

![Figure 6](image-url)
release of more IL-1β. However, intervening during the initiation phase is not practical, as patients are diagnosed only after an AAA has developed. Importantly, we demonstrate that IL-1β is also required for AAA progression, as evidenced by the attenuation of AAs with delayed anakinra treatment. By competitively binding IL-1R, anakinra halts the cycle of IL-1β production and AAA progression.

The IL-1β pathway is an attractive target for AAA treatment for 2 reasons: (1) disruption of the IL-1β pathway blocks the inflammatory pathway contributing to AAA formation and (2) neutralization of IL-1β appears safe in humans. Although there are multiple factors that contribute to AAA development, inflammation is a prominent feature. IL-1β neutralization has recently been shown to have widespread benefit in many autoimmune inflammatory conditions, including gout, type 2 diabetes mellitus, autoimmune pericarditis, and rheumatoid arthritis.

Similar to our finding of direct correlation between IL-1β levels and aortic dilation in mice, IL-1β levels correlate with disease severity of rheumatoid arthritis. Antagonism of the IL-1β signaling appears to be safe in humans for multiple years. Furthermore, multiple pharmacological drugs that disrupt IL-1β signaling are already approved.

IL-1R antagonism in experimental AAs has been previously evaluated by Hingorani et al, who treated WT rats with intraperitoneal anakinra 100 mg/kg every 8 hours after elastase perfusion. On aortic harvest 6 days after perfusion, there was no effect of anakinra on aortic diameters. In our model, significant decreases of aortic dilation were seen only after day 7. Up to day 7, aortic dilation of IL-1β KO, IL-1R KO, and WT mice were similar. Therefore, although the mice were treated with adequate doses of IL-1R antagonist, differences may not have been seen because of the brief time course of treatment. Consequently, our study is the first to indicate the critical role of IL-1β in AAA formation and the potential therapeutic option of IL-1R antagonism to prevent AAA development.

The effects of IL-1β antagonism are unique from general immune suppression. Lindeman et al reported a case of a patient with a rapid AAA expansion after kidney transplant with posttransplant immunosuppression regimen of prednisone, cyclosporine, and mycophenolate. In this patient, the abdominal aortic diameter increased from 3.4 cm to 7.0 cm over 30 months (14 mm/year), significantly more rapid than the expected rate of 3 mm/year. Histological evaluation of the AAA demonstrated an absence of T-cells, B-cells, and neutrophils; however, macrophages and mast cells were abundantly present and did not appear affected by immunosuppression. Macrophage infiltration has been linked with IL-1β, and we have shown IL-1β neutralization is associated with minimal macrophage staining. Furthermore, Dinarello has observed that many patients with autoimmune inflammatory diseases that were typically not responsive to immunosuppressive treatments were often responsive to IL-1β blockade. Therefore, we believe that IL-1β antagonists function differently from traditional immunosuppressive agents.

There are several limitations of the present study. Although a relatively acute model, the elastase perfusion model closely models human AAA and has contributed much of the existing knowledge of AAA pathogenesis. The murine elastase model and human samples have numerous commonalities, including elastin degradation, macrophage infiltration, inhibition of smooth muscle cell proliferation, and increased collagen turnover along with increased MMPs and inflammatory cytokines, including IL-1β. IL-1β is produced in a precursor form and must be cleaved to form active IL-1β. Although ELISA and immunohistochemistry antibodies are directed at active IL-1β, the precursor form may be bound because the precursor IL-1β contains the same amino acid sequence as the active form. However, Herzyk et al demonstrated that commercially available ELISAs do not bind the precursor form well, and therefore mostly represent the active form of IL-1β. We demonstrated that anakinra was effective in the prevention and treatment of experimental AAA formation at a dosage of 100 mg/kg day. This dose exceeds the dosage approved for patient use, which is approximately 1 mg/kg day. The excess anakinra required to demonstrate an effect is not unique to this study and has been similarly shown by other groups evaluating anakinra in murine models. As anakinra is recombinant human IL-1R antagonist, there may be a decreased specificity for mouse IL-1R that may explain the need for higher doses. In human diseases, decreased inflammatory markers and symptomatic relief are seen with doses of 1 mg/kg per day. Although IL-1R antagonism effectively inhibited experimental AAA formation over the 14-day model, patients may require long-term IL-1β blockade for AAA treatment. Fleischmann et al showed that long-term daily use of IL-1R antagonists by humans was well tolerated for up to 36 consecutive months.

Further evaluation of the protective effects of disruption of the IL-1β pathway in nonmyeloid-derived cells is required. Although the mechanism of IL-1β has been well studied, the cell types critical for AAA formation are undetermined and complex. We demonstrated that IL-1β colocalized with aortic smooth muscle cells early in AAA formation. Additionally, the protective effects of IL-1R genetic deletion were more apparent in IL-1R KO host animals receiving WT bone marrow rather than WT mice receiving IL-1R KO bone marrow. Thus, the protective effects of IL-1R gene deletion in AAA are mostly derived from nonmyeloid-derived cells. In the aorta, the most common nonbone marrow-derived cell is the vascular smooth muscle cell. Vascular smooth muscle cells have tremendous plasticity and respond to local environmental factors to change their phenotype from a differentiated, contractile state to a more proliferative/inflammatory state. We have previously shown that aortic smooth muscle cell phenotypic switching occurs during experimental AAA formation with a coordinated repression of smooth muscle cell marker genes and increased production of MMPs. Future studies are needed to investigate the IL-1β pathway in aortic smooth muscle cells during AAA pathogenesis.

Protection from AAA formation in IL-1R KO mice and WT mice treated with recombinant IL-1R antagonist was similar to IL-1β KO mice, suggesting that the protective effects of anakinra are IL-1β-derived. Additionally, increasing anakinra dosages correlated with decreasing IL-1β levels and decreasing aortic diameter, implying that the benefit of IL-1R antagonism relates to IL-1β inhibition. However, IL-1R also binds IL-1α, which is also upregulated in the murine elastase AAA model.
Therefore, additional studies are required to evaluate the role of IL-1α during AAA formation, as it is possible that receptor deletion or receptor antagonist may affect IL-1α signaling. Thorough investigation of IL-1α is beyond the scope of this study.

In conclusion, we have demonstrated that IL-1β signaling is essential for experimental AAA formation. IL-1β is increased early in AAA formation and stimulates a proinflammatory state typical for AAAs. Deletion of IL-β or its receptor prevents activation of inflammation and reduces downstream proinflammatory cytokines. IL-1β and IL-1R may be useful targets for potential pharmacological inhibition or treatment of AAAs, as neutralization of IL-1β signaling with anakinra prevents both AAA initiation and progression. Disruption of IL-1β signaling deserves further evaluation in large animal models or patient trials for AAA treatment.

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Disclosures

None.

References


Genetic and Pharmacologic Disruption of Interleukin-1β Signaling Inhibits Experimental Aortic Aneurysm Formation
William F. Johnston, Morgan Salmon, Gang Su, Guanyi Lu, Matthew L. Stone, Yunge Zhao, Gary K. Owens, Gilbert R. Upchurch and Gorav Ailawadi

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At the time of harvest, aortic tissue was perfused with 4% paraformaldehyde (PFA) solution and then placed in 4% PFA overnight. Tissues were rinsed with PBS (3 x 5 minutes), transferred to 70% ethanol, and then embedded in paraffin. Immunohistochemistry was performed on WT mice perfused with elastase or saline for IL-1β using a mouse IL-1β antibody (R&D Systems). Additionally, aortas from IL-1β KO, IL-1R KO and WT mice were examined with immunohistochemistry for macrophages, neutrophils, and elastin using a mac-2 antibody (Cedarlane), rat anti-mouse Ly-6B.2 antibody (AbD Serotec, Kidlington, UK), and Verhoeff-Van Gieson (VVG) stain, respectively. To evaluate the distribution of IL-1β following treatment with the IL-1R antagonist anakinra, aortic sections of the treatment groups were treated with IL-1β antibody (H:153, Santa Cruz Biotechnology, Santa Cruz, CA).

**Supplemental Tables:**

**Table I:** Gene sequences for PCR primers.

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Supplemental Figure I: Reproducibility of the method of aortic measurements with (A) inter-observer correlation of aortic dilation between original % aortic dilation compared to % dilation calculated from blinded remeasurements, (B) Bland-Altman plot comparing the difference (%) between the original and blinded aortic dilations compared to the average aortic dilation, (C) correlation of individual aortic diameter measurements of both control sections and maximal aortic diameters between original and blinded measurements, and (D) Bland-Altman plot of aortic diameter measurements comparing difference in individual measurements (mm) with average aortic diameter.
Supplemental Figure II: Study analysis based on maximal aortic dilation (mm) of the study groups (A-E). Actual aortic diameter of the internal control section shown in (F). * = p<0.0005 vs. WT Elastase, † = p<0.05 vs. Vehicle, ‡ = p<0.005 vs. vehicle, ¶ = p<0.05 vs. WT → WT, # = p<0.005 vs. IL-1R KO → WT.
Supplemental Figure III: Immunohistochemistry for IL-1β (brown stain) in A) healthy human aorta and B) human abdominal aortic aneurysm using IL-1β antibody (black arrows).
**Supplemental Figure IV:** (A) Aortic dilation at baseline (day 0), day 3, day 7, and day 14 of C57Bl/6 mice that underwent aortic isolation and perfusion with elastase or saline. (B) Sample photos are shown of the murine infrarenal abdominal aorta at baseline prior to perfusion, as well as at 14 days following elastase or saline perfusion.
Supplemental Figure V: IL-1α gene expression over the course of the murine elastase AAA model (green) compared to saline vehicle alone (gray).
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