Interleukin-1β Is Crucial for the Induction of Coronary Artery Inflammation in a Mouse Model of Kawasaki Disease

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Background—Kawasaki disease (KD) is the most common cause of acute vasculitis and acquired cardiac disease in US children. Untreated, children may develop coronary artery aneurysms, myocardial infarction, and sudden death as a result of the illness. Up to a third of KD patients fail to respond to intravenous immunoglobulin, the standard therapy, and alternative treatments are being investigated. Genetic studies have indicated a possible role for interleukin (IL)-1β in KD. We therefore explored the role of IL-1β in a murine model of KD.

Methods and Results—Using an established mouse model of KD that involves injection of Lactobacillus casei cell wall extract (LCWE), we investigated the role of IL-1β and caspase-1 (activated by the inflammasome and required for IL-1β maturation) in coronary arteritis and evaluated the efficacy of IL-1 receptor antagonist as a potential treatment. LCWE-induced IL-1β maturation and secretion were dependent on the NLRP3 inflammasome in macrophages. Both caspase-1-deficient and IL-1 receptor-deficient mice were protected from LCWE-induced coronary lesions. Injection of recombinant IL-1β into caspase-1-deficient mice restored the ability of LCWE to cause coronary lesions in response to LCWE. Furthermore, daily injections of the IL-1 receptor antagonist prevented LCWE-mediated coronary lesions up to 3 days after LCWE injection.

Conclusions—Our results strongly suggest that caspase-1 and IL-1β play critical roles in the development of coronary lesions in this KD mouse model, blocked by IL-1 receptor antagonist. Therefore, anti–IL-1β treatment strategies may constitute an effective, more targeted treatment of KD to prevent coronary lesions. (Circulation. 2012;125:1542-1550.)

Key Words: Kawasaki disease ■ myocarditis ■ Interleukin-1 receptor antagonist protein ■ Interleukin-1 beta ■ vasculitis

Kawasaki disease (KD) is an acute febrile illness and systemic vasculitis of unknown origin that affects predominantly children <5 years of age. Twenty-five percent of untreated patients with KD will develop acute coronary arteritis, often with the development of coronary artery aneurysms; accordingly, KD represents the leading cause of acquired heart disease among children. Although its origin is unclear, KD involves systemic inflammation with a distinct predilection for the coronary arteries. The resulting coronary arteritis in KD is characterized histologically by inflammatory cell infiltration and destruction of extracellular matrix, especially elastic tissue in vascular media, with resultant coronary artery aneurysm formation. Mortality in KD virtually always results from ischemic myocardial disease. The risk is highest in the first year after KD, but myocardial infarction has been increasingly reported in young adults with missed KD. Long-term cardiovascular complications among survivors of childhood KD have been reported with increasing frequency. The limited understanding of the etiologic agent(s) and cellular and molecular pathology of vasculitis continues to thwart development of more efficacious treatments or cures.

Current treatment with a single dose of intravenous immunoglobulin (IVIG) is effective in reducing the incidence of coronary artery aneurysms and has been the gold-standard treatment to prevent coronary lesions in children with KD. However, up to a third of children will have persistent or recrudescence fever after initial IVIG treatment (IVIG nonresponders) and are at increased risk for development of coronary abnormalities. The optimal therapy for these IVIG nonresponders remains controversial, and agents used for secondary or rescue therapy vary among centers. Anti–tumor necrosis factor-α (TNF-α) monoclonal antibodies (mAbs), including infliximab, have potent anti-inflammatory effects and are cur-
rently undergoing evaluation in large-scale clinical trials. When retreatment of IVIG-resistant KD patients is required, infliximab resolves fever more quickly and decreases the number of days of hospitalization compared with IVIG treatment.17

To further explore the pathogenesis of vasculitis in KD, we used the Lactobacillus casei cell wall extract (LCWE)–induced mouse model of coronary arteritis, a well-established model that histopathologically mimics the coronary arteritis of KD.18–20 Most important, this experimental mouse model has proven to be useful in duplicating or predicting human treatment responses because IVIG and anti–TNF-α mAbs were found to be effective in preventing coronary lesions in an LCWE-induced mouse model.19,21

KD is an inflammatory disease that leads to generalized vasculitis. IL-1β is a prototypic proinflammatory cytokine considered the gatekeeper of inflammation, and its induction and release are independent of TNF-α. IL-1β has been shown to be upregulated in patients who have failed standard therapy with IVIG. Pro–IL-1β is biologically inactive until it is enzymatically cleaved by the caspase-1 complex (inflammasome) to generate the bioactive IL-1β protein, which is then secreted.22 IL-1β signaling is mediated through the type I IL-1 receptor. Additionally, the IL-1β receptor antagonist (IL-1Ra), an endogenous molecule, can bind the IL-1β receptor and prevent normal IL-1 signaling.23 Recombinant IL-1Ra (Anakira) has been approved for the treatment of many inflammatory diseases such as rheumatoid arthritis.24 It has been suggested that IL-1β plays a critical role in chronic inflammatory diseases such as atherosclerosis, gout, and diabetes mellitus and more recently has possibly been linked to KD.24–26

Several clinical clues exist to suggest that IL-1β may play an important role in KD. Maury et al27 reported that the serum level of IL-1β was significantly increased in KD patients compared with age-matched healthy control subject. Popper et al28 reported gene expression patterns of KD patients, demonstrating that acute KD was characterized by increased relative abundance of gene transcripts associated with innate immune and proinflammatory response, including the IL-1β gene. Furthermore, several reports now show that IVIG nonresponders have increased IL-1β gene expression and diminished IL-1Ra expression.29 Furthermore, although the exact mechanism by which IVIG is effective in preventing coronary artery lesions in KD patients is unknown, several studies have determined that IVIG is associated with a reduction in IL-1β secretion in KD patients (in vivo).30,31 and IVIG has been shown to downregulate IL-1 and to upregulate IL-1Ra production in vitro.32,33 Collectively, these observations strongly suggest that IL-1β may play an important role in KD.

We previously used MyD88 and toll-like receptor 2 (TLR2) knockout mice to show that TLR signaling is critically involved in LCWE-induced coronary lesions in the KD mouse model.34 In addition to being the adaptor molecule for TLR2 signaling, MyD88 is required for both the formation of pro–IL-1β (via nuclear factor-κB activation) and for IL-1β signaling. From these clinical and experimental observations, we hypothesized that IL-1β plays a key role in KD patients. Accordingly, we investigated the specific role of IL-1β and the effectiveness of an anti–IL-1 therapeutic agent, IL-1R antagonist, in an LCWE-induced mouse model of KD. Here, we report that LCWE does not induce coronary arteritis in caspase-1–deficient and IL-1R–deficient mice, indicative of the key role that IL-1β plays in the pathogenesis of coronary lesions in the KD mouse model. We also observed that IL-1Ra effectively blocks LCWE-induced vasculitis, coronary lesions, and myocarditis in this model, suggesting that novel treatments with inhibitors of IL-1β could provide effective and more targeted therapies and could prevent the cardiac complications in human KD.

**Methods**

**Mice**

Wild-type (WT) C57BL/6, type I IL-1R (Il1r1)−/−, and Ifn-γ−/− mice (all on C57BL/6 background) were purchased from The Jackson Laboratory (Bar Harbor, ME). Castl/g−/− mice were obtained from Dr R.A. Flavell (Yale University, New Haven, CT). Il17a−/− mice were obtained from Dr Y. Iwakura (University of Tokyo, Tokyo, Japan). Nlrp3−/− and Asc−/− mice were obtained from Dr K.A. Fitzgerald (University of Massachusetts, Worcester). All animals were housed under specific pathogen-free conditions at the animal center of the Cedars-Sinai Medical Center. Experiments were conducted under approved Institutional Animal Care and Use Committee protocols. The number of animals used in various experiments ranged from 5 to 12 in each group as specified in the figure legends.

**Reagents**

Lipopolysaccharide from Escherichia coli (InvivoGen, San Diego, CA), recombinant IL-1Ra (Anakirina-Kineret, Amgen), recombinant mouse IL-1β (Sigma, St. Louis, MO), human TNF-α mAb (Infliximab, Merck), and ATP (Sigma, St. Louis, MO) were used in these studies. IL-1Ra was used at 25 mg/kg or 500 μg per mouse given intraperitoneally. The dose was based on several published studies and pilot dose-dependent studies that we have done. Human TNF-α mAb was used at 10 mg/kg or 200 μg per mouse given intraperitoneally, a dose that was based on other published studies.

**Preparation of LCWE**

LCWE (ATCC 11578) was prepared as previously described.34 In brief, L. casei were grown in Lactobacillus of Man, Rogosa, and Sharpe broth (Difco) for 48 hours, harvested, and washed with PBS. The harvested bacteria were disrupted by 2 packed volumes of 4% SDS/PBS during overnight. Cell wall fragments were washed 8 times with PBS to remove any residual SDS. The SDS-treated cell wall fragment was sonicated for 2 hours with a 3/4-in horn and a garnet probe, washed twice with PBS to remove any residual SDS. The cell wall fragments were then spun for 20 minutes at 12 000 rpm and 4°C. The supernatant was collected and the pellet was discarded. The total rhamnose content of the cell wall extract was determined by a colorimetric phenol-sulfuric assay as described previously.18

**KD Mouse Model and Inflammation Scores for Coronary Arteritis, Aortitis, and Myocarditis**

Mice 4 to 5 weeks of age were injected intraperitoneally with 250 μg LCWE (total rhamnose amount as determined above) or PBS. Mice were euthanized and hearts were removed at day 7 or 14 and embedded in optimal cutting temperature compound for histological examination. After a cut was made through the aortic root, coronary artery lesions, aortic root vasculitic lesions (aortitis), and myocardial inflammation were identified in serial sections (7 μm) stained with hematoxylin and eosin or elastin/collagen staining. Only sections that showed the second coronary artery branch separating from the aorta were analyzed. Histopathological examination and inflammation severity scoring of the coronary arteries, aortic root vasculitis, and myocarditis were performed by a coronary pathologist blinded to the genotypes or experimental groups (M.F.). KD lesions were assessed with the following scoring system: 0 = no inflammation,
1=rare inflammatory cells, 2=scattered inflammatory cells, 3=diffuse infiltrate of inflammatory cells, and 4=dense clusters of inflammatory cells. Multinuclear cells were indicative of acute inflammation; mononuclear cells reflected chronic inflammation. The aortic root was evaluated for severity of aortitis, and cross sections of the coronary artery were evaluated for severity of coronary artery inflammation; combined, the 2 scores generated a severity score that we called vessel inflammation score. Myocardial inflammation score was described as follows: 0=no myocardial fibrosis, 1=very minimal focal subepicardial interstitial fibrosis just infiltrating beneath epicardial fat, 2=mild subepicardial interstitial fibrosis infiltrating deeper into the subepicardial myocardium, 3=multifocal subepicardial interstitial fibrosis, and 4=replacement fibrosis. The incidence rate was evaluated by the presence of any coronary, aortic, or myocardial inflammation score of ≥1.

**Measurement of Body Temperature**

The rectal body temperature of each individual mouse was measured 3 times a day at the same time of day by a digital thermometer (PRT-03), and the average value was calculated.

**Preparation of Bone Marrow-Derived Macrophages**

Femurs were flushed and bone marrow cells were cultured in RPMI-1640 medium containing 10% FBS and 20% L929 cell–cultured medium for 7 days. Cells were washed with PBS and nonadherent cells were removed; adherent cells were then collected and seeded in a 96-well plate 1 day before stimulation. Cells were treated with LCWE for 12 hours, and the culture supernatants were collected for measurement of various cytokines.

**Cytokine Measurement**

The cytokine concentrations in the plasma or culture supernatants of IL-1β, TNF-α (eBioscience), prostaglandin E2 (PGE2), and pro- 

**Statistical Analysis**

Results are reported as mean±SE. All data were analyzed with the Prism 4.03 statistical program. To compare differences in serum cytokine levels, the 2-tailed Student t test (at 95% confidence interval) was used to compare unpaired samples between experimen-

**Results**

**Primary Bone Marrow-Derived Macrophages Secrete TNF-α, IL-1β, and PGE2 in Response to LCWE Stimulation**

To investigate the LCWE-induced inflammatory responses in vitro from macrophages, we isolated primary bone marrow macrophages from WT mice and stimulated them with 10 μg/mL LCWE for 12 hours. We measured the levels of TNF-α, IL-1β, and PGE2 in the supernatants by ELISA because all of these cytokines have been associated with KD.17,27,35 As expected, LCWE induced the production of TNF-α in bone marrow macrophages (Figure 1A). Interestingly IL-1β and PGE2 were also induced in bone macrophages by LCWE (Figure 1A), indicating a possible role for them in the LCWE KD mouse model.

**LCWE Induces IL-1β Release in Macrophages via NLRP3- and ASC-Dependent Inflammasome**

To investigate how LCWE induces IL-1β in macrophages, bone marrow macrophages were isolated from WT, Nlrp3+/−, and Asc−/− mice and stimulated with 10 μg/mL LCWE. For caspase-1 to process pro-IL-1β into mature IL-1β, caspase-1 needs to be activated by one of the multimeric protein complexes known as inflammasomes. Inflammasomes require 2 signals for activation: signal 1 (nuclear factor-κB driven, typically though
TLR signaling), which induces the production of pro–IL-1β, and signal 2, which activates the inflammasome complex. One such inflammasome, the NLRP3 inflammasome, is activated by many diverse stimuli (second signal) such as extracellular ATP, bacterial infections, and various danger signals. We therefore investigated whether LCWE–induced IL-1β secretion also used this pathway (NLRP3). We observed that LCWE induced IL-1β secretion in an NLRP3- and ASC-dependent manner (P<0.001; Figure 1B), whereas TNF-α secretion was not affected by NLRP3 or ASC deficiency (Figure 1C). ASC is another component of the inflammasome complex that is required for activation of caspase-1. These data indicate that LCWE activates the NLRP3 inflammasome to induce IL-1β secretion and that LCWE provides both signals 1 and 2 for inflammasome activation.

Caspase-1 Deficiency Protects Mice From LCWE-Induced Vasculitis and Coronary Lesions

To test the functional role of inflammasome activation and IL-1β secretion in LCWE-induced coronary artery inflammation, caspase-1−/− (Casp1−/−) mice were injected with LCWE and the hearts were harvested at day 14. WT mice displayed pronounced vasculitis with acute and chronic cellular infiltration, elastin disruption in the aorta, and intense concentric inflammation around the coronary arteries approaching occlusion. In contrast, Casp1−/− mice showed clean, open coronary arteries with no vasculitis and intact elastin structure in the aorta (Figure 2A and 2B). The total vessel inflammation score was significantly lower in Casp1−/− mice compared with WT mice (P<0.001; Figure 2C). The incidence of vascular lesion (Figure 2D) was significantly diminished in Casp1−/− mice compared with WT mice (P<0.001). We also assessed the effects of caspase-1 activity on LCWE–induced myocardial inflammation and found that Casp1−/− mice had significantly reduced scores compared with WT mice (P<0.05; Figure 2E). These results demonstrate that caspase-1 activity is required for LCWE-induced coronary artery inflammation and suggest that the NLRP3 inflammasome is required for this process.

Recombinant IL-1β Reconstitutes the Development of Coronary Lesions in Caspase-1–Deficient Mice

To verify that caspase-1 activation exerted its protective role via activating IL-1β secretion (as opposed to IL-18, another inflammasome–induced cytokine), Casp1−/− mice were injected with recombinant IL-1β (10 ng) or PBS from day 0 to 5 after LCWE injection. IL-1β injection restored LCWE-induced coronary lesions in Casp1−/− mice (Figure 3A–3C). The vessel inflammation score was significantly increased in Casp1−/− mice that received IL-1β compared with PBS-treated Casp1−/− mice.
mice ($P<0.05$; Figure 3B). IL-1β daily injection alone was not sufficient to induce the coronary lesion in Casp1−/− mice without LCWE (data not shown). These results indicate that IL-1β activation via caspase-1 plays a key role in LCWE-induced coronary artery inflammation and suggest that IL-18 plays little to no role in the lesion development.

**IL-1R–Deficient Mice Are Protected From LCWE-Induced Coronary Lesions**

To define the role of IL-1β in the LCWE-mediated KD mouse model, we next investigated IL-1R−/− mice. IL-1R−/− mice were significantly protected from LCWE-induced coronary lesions (Figure 4A and 4B) in that no lesions were detected in IL-1R−/− mice compared with WT mice ($P<0.001$; Figure 4C). These results demonstrate that IL-1β signaling plays a critical role in the development of LCWE-mediated coronary arteritis.

**IL-1Ra Blocks LCWE-Induced Coronary Lesions**

We next investigated whether IL-1Ra (Anakinra) could block LCWE-induced coronary lesions. IL-1Ra was injected (500 μg IP) daily into C57BL/6 mice from 1 day before LCWE injection to day 5. The mice were euthanized on day 7, and the hearts were harvested for analysis. We observed that IL-1Ra significantly blocked LCWE-induced coronary lesions (Figure 5A–5C) and elastin disruption (data not shown). The overall vascular inflammation score was significantly decreased in the IL-1Ra–treated group compared with the PBS-treated control group ($P<0.001$; Figure 5B). The incidence of vasculitis was significantly decreased in IL-1Ra–treated mice compared with PBS-treated controls (9 of 9 versus 1 of 9; $P<0.01$; Figure 5C). The myocardium inflammation score was also significantly reduced in IL-1Ra–treated mice compared with control mice ($P<0.001$; Figure 5D). To compare these results with the efficacy of anti–TNF-α mAb treatment, we injected a different group of mice with 200 μg human anti–TNF-α mAb (Infliximab) intraperitoneally once on the same day (day 0) as LCWE injection and harvested the hearts on day 7 as above. Anti–TNF-α mAb was also able to inhibit LCWE-induced vasculitis, as measured by vascular inflammation score (Figure 5A and 5B), and incidence of coronary lesions (Figure 5C) but not myocarditis (Figure 5D) compared with PBS control and rat IgG control. IL-1Ra treatment showed a strong trend toward more effective inhibition (89% inhibition, 8 of 9 mice protected) for the incidence of coronary lesions compared with anti–TNF-α mAb (56% inhibition, 5 of 9 mice protected; Figure 5C). IL-1Ra also provided a strong trend toward more effective protection for LCWE-induced myocarditis compared with the anti–TNF-α mAb group (Figure 5D). Because IL-1Ra was able to almost completely prevent coronary lesion formation when given at the same time as the LCWE injection and throughout the LCWE

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

**Figure 4.** Interleukin-1 receptor (IL-1R)–deficient mice are protected from Lactobacillus casei cell wall extract (LCWE)–induced vasculitis and coronary arteritis. Wild type (WT; A) and Il1r1−/− (B) mice were administered LCWE intraperitoneally, and their hearts were harvested on day 14. Hematoxylin and eosin staining was performed, and representative sections are shown (A and B). Scale bar=250 μm. C, Incidence was evaluated by use of the Fisher exact test ($n=5$ or 9). A value of $P<0.05$ was considered statistically significant.

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

**Figure 5.** Interleukin-1 receptor antagonist (IL-1Ra) protects against Lactobacillus casei cell wall extract (LCWE)–induced vasculitis, coronary arteritis, and myocarditis. After LCWE injection, wild-type (WT) mice were administered 500 μg IL-1Ra intraperitoneally daily (day 1–5), 200 μg human tumor necrosis factor-α (TNF-α) monoclonal antibody (mAb; once on day 0), or the same volume of PBS for control, and hearts were harvested at day 7 for analysis. Representative hematoxylin and eosin–stained sections are shown (A). Scale bar=250 μm. Heart vessel inflammation score (B), incidence (C), and myocardium inflammation score (D) were evaluated for each group as mentioned in Methods. Data shown are mean±SE and were compared by the Kruskal-Wallis with the Dunn post hoc test (B and D) and Fisher exact test for incidence (C; $n=9$). A value of $P<0.05$ was considered statistically significant.
protocol, we next investigated whether IL-1Ra could still inhibit coronary arteritis when given after LCWE administration. IL-1Ra treatment was administered as before except with a varying starting point relative to LCWE injection as described in Figure 6A. We observed that IL-1Ra significantly inhibited LCWE-mediated coronary lesions even if treatment was delayed up to 3 days after LCWE injection (Figure 6B and 6C). These results suggest that inhibition of IL-1β signaling even several days after the injection of the LCWE is effective in preventing coronary artery vasculitis in this mouse model of KD.

**LCWE-Induced KD Mouse Model Is Associated With Body Temperature Elevation and Increased Circulating PGE₂ and Pentraxin 3 Levels**

IL-1β is an important pyrogenic cytokine that may be associated with clinical symptoms of KD patient. One hallmark of KD is the presence of high fever for many days. Therefore, we measured daily rectal body temperature after LCWE injection in WT mice. LCWE-injected mice displayed significantly increased body temperature compared with the PBS-injected control group (P<0.05 to P<0.001; Figure 7A). Additionally, we measured serum level of pentraxin 3 (a marker of systemic inflammation) and PGE₂ (known to be elevated in KD patients) during LCWE-induced coronary arteritis. Similar to patients with KD, serum PGE₂ and pentraxin 3 levels at day 7 or 14 were significantly increased in LCWE-injected WT mice compared with PBS-treated or naïve WT mice (P<0.001; Figure 7B–7E). Consistent with
the inhibition of coronary lesions, circulating levels of PGE$_2$, and pentraxin 3 were significantly decreased in caspase-1$^{-/-}$ mice (day 14; $P<0.05$ and $P<0.001$; Figure 7B and 7D) and IL-1Ra-treated mice (day 7; $P<0.05$ and $P<0.01$; Figure 7C and 7E) compared with extract-injected WT mice. These results suggest that, similar to the systemic inflammation seen in KD patients, the LCWE-mediated KD mouse model, in addition to focal coronary lesions, also induces systemic inflammatory changes.

Discussion

KD is now recognized as the leading cause of acquired heart disease in children in the United States and developed world. The underlying origin and mechanisms leading to vessel inflammation, coronary artery lesions, and aneurysms, which are the hallmarks of KD, remain largely unknown. We show in this study that IL-1$\beta$ signaling is critically required for the development of LCWE-induced vasculitis. Whereas the caspase-1–deficient mice were clearly defective in their response to LCWE, caspase-1 is known to cleave and activate multiple cytokines, including IL-1$\beta$ and IL-18. However, given the total lack of lesions found in IL-1R1–deficient mice and the ability of recombinant IL-1$\beta$ to reconstitute lesion development in Casp1$^{-/-}$, mice, it is clear that IL-1$\beta$ plays a critical role in LCWE-induced coronary arteritis or a KD mouse model.

We have previously shown that the LCWE-induced KD mouse model is dependent on both innate and adaptive immunity and that both the TLR2/MyD88 signaling pathway and the presence of T cells are required for coronary lesions to develop. In this study, we now show that IL-1$\beta$ is critically important and required in the KD mouse model and that IL-1Ra treatment can effectively prevent LCWE-induced coronary lesions even if treatment is started up to 3 days after the extract injection. Given that T cells are required for this mouse model and that a previous study found that interferon-γ–deficient mice are not protected from developing coronary lesions after LCWE injection, we hypothesized that IL-17A may play a role in this model. Additionally, several studies have shown that IL-1$\beta$ signaling can drive Th17 skewing. However, to our surprise, we found that IL-17A–deficient mice developed robust coronary lesions in response to LCWE (Figure I in the online-only Data Supplement). One possibility is that T cells accumulating in the coronary lesions induce a strong chemokine induction, recruiting large numbers of monocytes, macrophages, and dendritic cells that secrete large amounts of inflammatory cytokines such as IL-1$\beta$.

It is important to acknowledge that although the LCWE-induced coronary arteritis mouse is a model for KD, it cannot be considered exactly similar to human disease because the etiologic agent for KD is yet to be discovered. However, there are striking similarities in the histopathology and kinetics of lesions between human KD and this LCWE-induced coronary arteritis mouse model of KD. The LCWE-induced mouse model of coronary arteritis appears to be unique in demonstrating not only acute myocarditis and coronary arteritis with aneurysm formation but also chronic scarring of the coronary arteries with the formation of stenotic segments, luminal obstruction, and evidence of coronary artery thrombosis. The LCWE-mediated KD mouse model has been studied for >35 years, and it has reliably predicted treatment responses to agents such as IVIG in humans with KD. The current gold-standard treatment of IVIG for KD has been shown to be efficacious in preventing LCWE-induced coronary lesions in the KD murine model. In that study, Myones et al reported 40% to 67% inhibition in the incidence of coronary lesions when human IVIG was given to LCWE-injected mice between days 3 and 5. This is very similar to the 55% protection in the incidence of coronary arteritis that we have observed in mice treated with human IVIG given intraperitoneally at the same time as LCWE (data not shown). Furthermore, the mouse model was also used to show that polyclonal rabbit antibody against murine TNF-α was able to suppress LCWE-mediated coronary lesions. This finding, among others, has been the basis for using anti–TNF-α mAb in treating KD in a number of KD patients and resulted in a larger ongoing clinical trial. In the present study, because we saw a very significant protection of coronary lesion formation and myocarditis with human IL-1Ra in this mouse model, we also wished to compare the efficacy of IL-1Ra with that of human anti–TNF-α mAb. Consistent with the earlier published data with polyclonal anti-murine TNF-α antibody, we also observed significant protection by human anti–TNF-α mAb in the LCWE-induced coronary arteritis in this KD model. There was a trend toward more effective inhibition of coronary lesion formation, inflammation severity score, and myocarditis score in the IL-1Ra–treated group compared with the anti–TNF-α mAb group.

Symptoms of KD include high fever for many days and systemic inflammation. We also show that the LCWE mouse model is associated with systemic inflammatory findings, including increased body temperature. Circulating levels of PGE$_2$, another pyrogen, in addition to IL-1$\beta$ were also elevated in the mouse model. We also observed that the KD mice had substantially elevated circulating levels of pentraxin 3, a molecule that is a local marker for vascular disease and coronary vasculitis.

Our findings suggest that the LCWE-induced mouse model of KD provides translational value to KD and posit the question, What role does IL-1$\beta$ play in human KD? Several studies have suggested that immune activation and the secretion of cytokines may contribute to the pathogenesis of KD. In particular, IL-1$\beta$ has been shown to increase significantly in patients during acute KD. In addition, several clinical and experimental clues strongly implicate the role of IL-1$\beta$ in KD. Previous studies have shown that IVIG influences the production and release of IL-1$\beta$ in KD patients and that IL-1$\beta$ polymorphisms associated with increased IL-1$\beta$ production are associated with IVIG resistance. Collectively, our findings and the emerging clinical and genetic data in KD patients and IVIG nonresponders suggest that IL-1$\beta$ may also play an important role in human disease and perhaps blocking it may provide a potential treatment in KD patients or certain subsets of IVIG nonresponders. This type of approach may fit well with the National Institutes of Health’s goal of personalized medicine.
Although treatment with IVIG is an effective therapy for KD, its mechanism of action is unknown, not all children respond, and optimal treatment of IVIG-refractory KD remains unclear. Identification of the cause of KD and a better understanding of the pathology of coronary lesions would greatly enhance efforts to improve targeted therapy and to prevent the cardiac complications of KD. In a large multicenter study, nearly 15% of KD patients required retreatment with IVIG because of failure to respond to initial treatment. Resistance to IVIG in children with KD has been reported to range between 7.8% and 38.2% and is associated with increased risk for coronary aneurysms. Therefore, there is an urgent need for alternative therapeutic modalities. Alternative treatments for patients with IVIG resistance are controversial. Additional IVIG treatments, steroids, anti–TNF-α mAbs, or other treatments have been used for IVIG-resistant patients with mixed results. Although anti–TNF-α mAb treatment in IVIG nonresponders has led to faster resolution of fever and fewer days of hospitalization, a retrospective study suggested that it did not reduce coronary aneurysms in patients. Therefore, our findings that IL-1β plays a significant role in LCWE-induced coronary lesions and that the blocking of its signaling by the IL-1Ra prevents lesion development support an innovative mechanistic insight into the cellular and molecular understanding of vasculitis and coronary arteritis in the LCWE-induced KD mouse model and may provide novel therapeutic strategies, including anti–IL-1β agents, to prevent the development of coronary lesions in KD patients.

Conclusions

We observed that IL-1β is critically involved in the LCWE-mediated coronary arteritis and myocarditis seen in the KD mouse model and that these lesions can efficiently be prevented by IL-1Ra treatment. These observations provide innovative mechanistic insights into the cellular and molecular understanding of vasculitis and coronary arteritis in the LCWE-induced KD mouse model and may provide novel therapeutic strategies, including anti–IL-1β agents, to prevent the development of coronary lesions in KD patients.

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Disclosures

None.

References


CLINICAL PERSPECTIVE

Kawasaki disease (KD) is now recognized as the leading cause of acquired heart disease in children in the United States and developed world. The underlying cause of KD and the mechanisms leading to vessel inflammation, coronary artery lesions, and aneurysms, which are the hallmarks of KD, remain largely unknown. Standard therapy with intravenous immunoglobulin (IVIG) effectively reduces the incidence of coronary arterial lesions, but 10% to 20% of patients with KD fail to respond to IVIG and thus show a high prevalence of coronary lesions. There is no definitive treatment recommendation for patients who fail to respond to an initial course of IVIG treatment. Rescue treatment for IVIG resistance includes additional IVIG dose(s), steroids, anti–tumor necrosis factor monoclonal antibody, and cyclosporine A, but these approaches have shown mixed results, and the effectiveness of these therapies remains controversial. Development of an optimal alternative for IVIG-resistant KD patients is now an urgent matter. Using a well-established KD mouse model, we found that interleukin-1β is critically involved in the development of coronary arteritis and myocarditis in KD mice and that these lesions can be prevented by treatment with an interleukin-1 receptor antagonist. These observations provide mechanistic insights into the cellular and molecular understanding of the vasculitis and coronary arteritis in the KD mouse model and suggest that anti–interleukin-1β agents might prevent the development of coronary lesions in KD patients. Our findings provide justification for future prospective multicenter clinical trials to determine the efficacy of therapies designed to block interleukin-1β–dependent signaling in children with KD.
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Supplemental Material

Supplemental Figure 1. IFN-γ and IL-17A deficiency failed to protect from LCWE induced coronary lesions. WT, IFN-γ−/− and IL17A−/− mice were administrated (i.p.) with 250 µg LCWE and their hearts were harvested on day 14, (n=10, 5, 12, respectively). (A-C) Representative H&E-stained sections are shown. The scale bar indicates 250 µm. All of the mice were developed vasculitis. (D) Incidence was compared by use of Fisher’s exact test. A probability value of $P<0.05$ was considered statistically significant.