TLR2 and MyD88 Contribute to \textit{Lactobacillus casei} Extract–Induced Focal Coronary Arteritis in a Mouse Model of Kawasaki Disease

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\textbf{Background}—Kawasaki disease is the most common cause of acquired cardiac disease and acute vasculitis in children, targets the coronary arteries, and can occasionally be fatal. The pathogenesis and the molecular mechanisms remain unknown. After injection of \textit{Lactobacillus casei} cell-wall extract (LCCWE), mice develop a focal coronary arteritis that histopathologically resembles Kawasaki disease, but the mechanism remains unclear. Here, we tested the hypothesis that signaling by Toll-like receptors (TLRs) through their key downstream adaptor molecule myeloid differentiation factor 88 (MyD88) is required for the cellular activation and coronary arteritis produced by LCCWE.

\textbf{Methods and Results}—Bone marrow–derived macrophages from TLR2- or MyD88-deficient mice were unresponsive to LCCWE-induced stimulation. In contrast, macrophages obtained from TLR4-deficient mice produced the same amount of interleukin-6 as macrophages from wild-type mice after stimulation with LCCWE. Intraperitoneal injection of LCCWE produced severe focal coronary arteritis in TLR4\textsuperscript{-/-} and C57BL/6 control mice but not in TLR2\textsuperscript{-/-} or MyD88\textsuperscript{-/-} mice. Collectively, these results indicate that LCCWE is a potent inducer of nuclear factor-\textbf{B} via TLR2 but not TLR4 and that this activation proceeds via the MyD88-dependent signaling pathway. In vivo studies suggest that TLR2\textsuperscript{-/-} mice are protected from LCCWE-induced coronary arteritis and that this protection is mediated through the adaptor molecule MyD88.

\textbf{Conclusions}—Our results provide important insights into the molecular signaling in this mouse model of coronary arteritis. We show here that LCCWE-induced coronary arteritis is dependent on intact TLR2 and MyD88 signaling. (\textit{Circulation.} 2005;112:2966-2973.)

\textbf{Key Words:} coronary disease ■ inflammation ■ pediatrics ■ aneurysm ■ immune system

Kawasaki disease (KD)\textsuperscript{1} is an acute vasculitis of unknown pathogenesis that affects predominantly children under 5 years of age and represents the leading cause of acquired heart disease among children in the United States.\textsuperscript{2-5} The coronary arteries are a specific target. It has been suggested that the inciting agent may be a bacterial superantigen,\textsuperscript{6} but this hypothesis is controversial.\textsuperscript{2} Recent reports implicate a human coronavirus as a potential pathogenetic agent.\textsuperscript{7,8} Coronary arteritis in KD is characterized histologically by inflammatory cell infiltration and destruction of the arterial media, especially elastic tissue in the media, with resultant coronary artery aneurysm formation. Subsequent thrombosis or, less commonly, rupture of diseased coronary vessels may occasionally be fatal. Although therapeutic strategies to downmodulate inflammation with intravenous immunoglobulin have reduced morbidity and mortality associated with KD,\textsuperscript{3,9,10} lack of a pathogenetic agent and incomplete understanding of the molecular mechanisms mediating the pathological changes of KD have hampered the development of targeted and more effective treatment options. Recently, studies implicating signaling via Toll-like receptors (TLRs) in vascular pathological conditions involving inflammation\textsuperscript{11,12} raise the possibility that TLR signaling may play a role in KD.

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Host defense depends critically on detection of invading pathogens. This task is largely performed by TLRs, which...
recognize specific molecular components common to a very broad range of microbes but rarely or never produced by the host itself.13-15 TLR signaling activates synthesis and release of inflammatory cytokines in part via nuclear factor (NF)-κB activation and coordinately controls both the innate and adaptive immune responses in a manner that is tailored to the nature of the pathogenic threat. Thus far, at least 10 human TLRs have been identified, recognizing specific pathogen-associated molecular patterns. The best-characterized of these receptors are TLR2 and TLR4, which have been implicated in host defense systems against Gram-positive and Gram-negative bacteria, respectively. TLR2 and TLR4 activate NF-κB through an adapter molecule, myeloid differentiation protein 88 (MyD88).16,17 Another adapter molecule for TLR4, called TIR domain–containing adaptor protein (TIRAP), associates with TLR4 and is critical for lipopolysaccharide (LPS)-induced dendritic cell maturation18,19 and essential for tumor necrosis factor-α and interleukin (IL)-6 production via MyD88-dependent TLR2 and TLR4 signaling pathways.20 Although most TLR signaling utilizes MyD88, some TLRs can activate gene targets using an MyD88-independent pathway that involves the adaptor proteins TRAM (TRIF-related adaptor molecule) and TRIF (TIR domain–containing adaptor inducing interferon [IFN]-β).21,22

After a single intraperitoneal injection of Lactobacillus casei cell-wall extract (LCCWE), mice develop a focal, localized coronary arteritis that is histopathologically similar to the coronary artery lesions found in human KD.23-25 Recent analyses of the original reports on this experimental animal model suggest the potential involvement of TLR-mediated signaling in the pathogenesis of KD. Several, but not all, inbred mouse strains demonstrate susceptibility to LCCWE. Intriguingly, C3H/HeJ mice, which have a point mutation in TLR4 resulting in defective macrophage signaling in response to LPS, fail to develop coronary arteritis.23-25 This finding suggests a potential role for TLR4 signaling in this animal model of coronary arteritis. However, C3H/HeJ mice are not a surrogate for TLR4-null mutant mice; hence, an unequivocal answer as to whether TLR4 signaling is involved in LCCWE-induced coronary arteritis has not yet emerged.

Here, we report a series of experiments using both murine and human cells that collectively demonstrate that LCCWE induces release of inflammatory cytokines through TLR2 but not TLR4 and that LCCWE activates NF-κB in a TLR2-dependent but TLR4-independent fashion. In vivo studies indicated that TLR4-deficient mice were not protected from LCCWE-induced coronary arteritis. In contrast, TLR2-deficient mice as well as MyD88-deficient mice were resistant to the induction of coronary arteritis by LCCWE compared with wild-type mice. Further investigations into molecular signaling pathways revealed that LCCWE activation of NF-κB requires the participation of the adaptor molecules MyD88 and TIRAP. Our results provide important new clues to the molecular signaling mechanisms of the LCCWE-induced coronary arteritis model of KD and specifically suggest that TLR2, MyD88, and TIRAP probably play key roles in the pathogenesis of this mouse model of coronary arteritis.

Methods

Cell Cultures

Immortalized human dermal microvascular endothelial cells (HMECs) (a generous gift from Dr F.J. Candal of the Centers for Disease Control and Prevention, Atlanta, Ga) were cultured in MCDB-131 medium supplemented with 10% heat-inactivated FBS, 2 mmol/L glutamine, and 100 μg/mL penicillin and streptomycin. Tissue culture reagents were purchased from Life Technologies. HEK 293 cell line cells were obtained from American Type Culture Collection and were cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 2 mmol/L glutamine, and 100 μg/mL penicillin and streptomycin. Murine aortic endothelial cells derived from C57BL/6 mice were isolated (>95% purity) and maintained as we have described elsewhere.14

Generation of Bone Marrow–Derived Macrophages From TLR4-, TLR2-, and MyD88-Deficient Mice

TLR4- and MyD88-deficient mice on a C57BL/6 background were kindly provided by Dr Shizuo Akira (Osaka University, Osaka, Japan), TLR2-deficient mice and C56BL/6 mice were purchased from Jackson Laboratory (Bar Harbor, Me). Bone marrow–derived cells were flushed from femurs and tibias of mice with medium (DMEM, 10% FBS, 2 mmol/L glutamine, 100 μg/mL penicillin and streptomycin) and washed 3 times. Cells were cultured for 3 days in medium supplemented with murine macrophage-colony stimulating factor (M-CSF) (Biosource International). On day 3, adherent cells were incubated with fresh M-CSF containing medium. On day 6, adherent cells were incubated with DMEM and used in experiments on day 8.

LCCWE Preparation and Other Reagents

The group B L casei wall fragments used were obtained from L casei (ATCC 11578) as described earlier.23 In brief, the bacteria were grown in L MRS broth (DIFCO), harvested by centrifugation during the exponential growth phase, and washed with phosphate-buffered saline (PBS), pH 7.4. The bacteria were then disrupted by overnight incubation in 4% sodium dodecylsulfate (SDS) in twice their packed volume. Cell-wall fragments were washed 8 times with PBS to remove any residual SDS. The cell-wall fragment preparation was then sonicated for 2 hours with a Heat Systems Ultrasonic W 373 sonicator with a three-quarter-inch horn and a garnet tip at maximum power. During sonication, the cell-wall fragments were maintained at 4°C. Then sonicated, the cell-wall fragments were spun for 1 hour at 20 000g at 4°C, and the supernatant was retained. The L casei cell-wall content of the supernatant was expressed as milligrams of total rhamnose per milliliter of PBS as determined by colorimetric phenol–sulfuric acid extraction technique.26 The endotoxin concentration of this preparation was <2.7 pg/mL as determined by the Limulus amebocyte lysate assay (Associates of Cape Cod). Bacterial lipopeptide (palmitoyl-Cys(Res)-2,3-di(palmitoloyloxy)-(propyl)-(Ala-Gly-OH) was purchased from Bachem. Purified, protein-free Escherichia coli K2335 LPS was obtained from Stefanie Vogel (University of Maryland, Baltimore).

IL-6 and MCP-1 Measurement by ELISA

Macrophages from TLR4+/+, TLR2−/−, MyD88−/−, and their control littermates or murine aortic endothelial cells were plated at 5000 cells per well in 96-well plates 24 hours before stimulation. The cells were treated with 10 ng/mL LPS and 1, 5, 10, and 15 μg/mL LCCWE or with 1, 5, 10, and 15 μg/mL LCCWE treated with proteinase K (Roche Applied Science). In brief, LCCWE was digested with proteinase K at a final concentration of 100 mg/mL for 30 minutes at 56°C and subsequently inactivated at 95°C for 5 minutes. Supernatants were harvested 24 hours after stimulation. IL-6 or monocyte chemotactic protein (MCP)-1 concentrations were measured by ELISA (BD Bioscience).
NF-κB Promoter Luciferase Activation in HMECs and HEK 293 Cells

HMECs were transiently transfected with an NF-κB promoter-luciferase construct as we described earlier. After overnight transfection, cells were stimulated for 6 hours with 50 ng/mL LPS, 2 μg/mL lipopeptide, or either 10 or 15 μg/mL LCCWE. Cells were then lysed, and luciferase activity was measured as described previously. Dominant negative cDNA constructs of MyD88 (DN-MyD88) or TIRAP (DN-TIRAP) were transiently transfected as described earlier. Data are shown as mean±SD of 3 or more independent experiments and are reported as a percentage of LPS-stimulated NF-κB promoter activity or relative luciferase activity.

Intraperitoneal Injection of L casei Cell-Wall Extract in TLR4−/−, TLR2−/−, MyD88−/−, and Wild-Type Mice and Evaluation of Cardiac Histology

A single intraperitoneal injection of group B LCCWE has been demonstrated to induce an intense inflammatory coronary arteritis in mice that histopathologically closely resembles the coronary vasculitis of KD, as we reported previously. We injected 8-week-old female TLR4−/− mice (n=5), MyD88−/− mice (n=5) (both backcrossed to C57BL/6 for at least 6 generations), TLR2−/− mice (n=10), and control C57BL/6 mice (n=16) with 500 μg of LCCWE in PBS or PBS alone intraperitoneally. Fourteen days later, mice were killed, and hearts were removed, perfused with PBS, and embedded in optimal cutting temperature (OCT) compound. Coronary arteries were identified in serial sections (6 μm) stained with hematoxylin and eosin. Blinded assessment of the histopathology of the coronary arteries and aortic root was performed, with particular emphasis on the region in proximity to the ostia of the coronary arteries.

Statistical Analysis

Results are reported as mean±SD. Experimental results were repeated at least in triplicate. Mean values were compared by use of Student’s t test to determine statistical differences between experimental groups. A probability value of P≤0.05 was considered statistically significant.

Results

LCCWE-Induced IL-6 Cytokine Secretion by Bone Marrow–Derived Macrophages Is TLR2 Dependent

We first investigated cytokine expression of bone marrow–derived macrophages from mice harboring null mutations of specific TLR pathways, TLR2 and TLR4, as well as the adaptor molecule MyD88. Primary bone marrow–derived macrophages from TLR4−/−, TLR2−/−, and MyD88-deficient mice and their wild-type controls were stimulated with LCCWE, LPS (a TLR4 ligand), or Pam3Cys (a synthetic TLR2 ligand). After 24 hours, the supernatants were harvested, and IL-6 release was measured. Macrophages from TLR2-deficient mice were unresponsive to LCCWE, whereas the response was intact in TLR2 wild-type macrophages (Figure 1A).

LCCWE Activates NF-κB in TLR2-Transfected HMECs

Human endothelial cells are one of the first lines of defense against invading microbial agents and actively participate in innate immune responses. We have demonstrated that HMECs express predominantly TLR4 and respond vigorously to TLR4 ligands such as LPS but do not express TLR2 and are unresponsive to a variety of TLR2 ligands unless they

Figure 1. LCCWE induces IL-6 secretion from bone marrow–derived macrophages via TLR2 but not TLR4. Bone marrow macrophages from TLR2 (A), MyD88 (B), and TLR4 (C) knockout mice and their wild-type controls were stimulated with LPS (10 ng/mL), Pam3Cys (2 μg/mL), or increasing concentrations of LCCWE for 24 hours as described in Methods. IL-6 response was measured by ELISA. Data shown are mean±SD of 1 representative experiment out of 3 independent experiments. N. D. indicates not detectable.
are transfected with exogenous TLR2.27 We used this in vitro experimental system to investigate the role of TLR2 and TLR4 in NF-κB activation by LCCWE. HMECs transfected with an NF-κB–promoter luciferase construct with and without cotransfection with exogenous TLR2 were stimulated with various concentrations of LCCWE, and their responses were measured by NF-κB luciferase activity. As expected, LCCWE did not stimulate NF-κB activation in native HMECs (Figure 2). LPS (50 ng/mL) did activate NF-κB in native HMECs, and this activation was blocked by overexpression of a dominant negative (DN) TLR4 or DN-TIRAP construct (Figure 2).

Next, we transiently transfected wild-type TLR2 construct into HMECs. TLR2-transfected HMECs showed a 10-fold increase in NF-κB activation on stimulation with LCCWE (Figure 3A). This observation further proves that TLR2 is necessary for NF-κB activation by LCCWE in HMECs. Next, we cotransfected wild-type TLR2 with DN-MyD88 or DN-TIRAP (Figure 3B). We observed a 70% and 90% decrease in LCCWE-induced NF-κB activity with DN-TIRAP and DN-MyD88, respectively, indicating that LCCWE induces NF-κB in a TLR2-, MyD88-, and TIRAP-dependent manner.

LCCWE Activates NF-κB in TLR2-Transfected HEK 293 Cells in an MyD88- and TIRAP-Dependent Manner

To further characterize the usage of TLRs by LCCWE, we used HEK 293 cells, which do not endogenously express TLR2 or TLR4/MD-2. To respond efficiently to LPS, TLR4 requires an accessory protein, MD-2.28 MD2 is a 20- to 30-kDa glycoprotein that binds to the extracellular domain of TLR4. To further investigate the role of TLR2 or TLR4 in LCCWE stimulation, 293 cells were cotransfected with NF-κB–luciferase and either TLR4 and MD-2 or TLR2 constructs and stimulated with either LPS, Pam3Cys, or LCCWE. Figure 4A shows that LCCWE does not activate NF-κB in HEK 293 cells expressing TLR4/MD-2. Conversely, there is a robust response of LCCWE-stimulated HEK 293 cells expressing TLR2, with a 30-fold increase in luciferase activity (Figure 4B). This further supports the fact that LCCWE activates NF-κB via TLR2 and not TLR4. HEK 293 cells were then cotransfected with TLR2 and dominant negative constructs of MyD88 and TIRAP (Figure 4C). There was an almost complete suppression of luciferase activity with the overexpression of the dominant negative constructs, confirming that LCCWE activation of NF-κB requires intact MyD88 and TIRAP signaling.

Pretreatment of LCCWE With Proteinase K to Degrade Proteins Did Not Alter MCP-1 Expression by Murine Aortic Endothelial Cells

Superantigens are antigenic proteins that can stimulate an unusually large proportion of T cells by binding to one part of the T-cell receptor.29,30 Proteins derived from a variety of bacteria and viruses possess superantigenic properties. A recent study suggested that LCCWEs may possess proteins with superantigenic activity.31,32 To evaluate this possibility, we digested our LCCWE preparation with proteinase K. Because proteinase K degrades proteins, and because superantigenic activity is protein dependent, this treatment should destroy any superantigenic activity in our LCCWE preparation.
tion. Murine aortic endothelial cells were stimulated with LCCWE (1, 5, or 10 μg/mL) or with proteinase K–digested LCCWE (1, 5, or 10 μg/mL). Proteinase K digestion had no effect on induction of MCP-1 release into the supernatant after 24 hours of treatment compared with ECs treated with LCCWE without previous proteinase K digestion (Figure 5). Coomassie blue stain confirmed the depletion of proteins in the digested LCCWE (data not shown). These results are consistent with the interpretation that the coronary arteritis produced by LCCWE is most likely independent of superantigenic proteins in the extract.

TLR2- and MyD88-Deficient Mice Are Protected From LCCWE-Induced Coronary Arteritis, Whereas TLR4-Deficient Mice Are Not

Intraperitoneal injection of LCCWE produced severe focal acute coronary arteritis in all 5 TLR4−/− mice, whereas 0 of 10 TLR2−/− and 0 of 5 MyD88−/− mice developed coronary arteritis (Table). Cardiac tissue from mice injected with PBS alone did not exhibit inflammation. Histological results obtained from mice 14 days after LCCWE injection are shown in Figure 6 and appeared qualitatively similar to those previously reported in this model.23–25 These results imply that the receptor that detects LCCWE is TLR2 but not TLR4. In brief, the coronary lesions consisted of a localized, nodular infiltrate of acute and chronic inflammatory cells localized to the most proximal portion of the coronary artery, with involvement of the ostium. The infiltrates were associated with destruction of the arterial media, with a proliferation of spindle cells in the intima. In some cases, the combination of inflammation and intimal proliferation resulted in complete occlusion of the arterial lumen. The inflammatory infiltrate was transmural, involving the adventitia, media, and intima of the arteries (Figure 6).

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Discussion

Here, we report in vitro and in vivo evidence that LCCWE uses MyD88 and TLR2 but not TLR4 to induce focal coronary arteritis in mice that histologically resembles the coronary artery changes observed in KD.23–25 Using bone marrow–derived macrophages from TLR2−/−, TLR4−/−, and MyD88−/− deficient mice, we demonstrate that LCCWE induces NF-κB–dependent cytokine release through TLR2 and not TLR4. We confirmed these observations in HEK 293 cells, which responded similarly to stimulation with LCCWE with activation of NF-κB only when transfected with TLR2 but not with TLR4 and MD-2. We then determined the role of the various adaptor molecules of the TLR signaling pathway in LCCWE-mediated NF-κB activation. Transient transfection of dominant negative constructs of MyD88 and TIRAP inhibited LCCWE-induced NF-κB activation, consistent with the interpretation that the effects of LCCWE were mediated by TLR signaling that utilizes the adaptor proteins MyD88 and TIRAP. In vivo experiments using TLR2−/−, MyD88−/−, or TLR4−/− deficient mice similarly indicated that LCCWE could induce coronary arteritis in TLR4−/− but not in TLR2−/− or MyD88−/− mice. The coronary arteritis in TLR4−/− mice appeared histopathologically similar to that seen in C57BL/6 wild-type mice, suggesting that TLR signaling instigated by LCCWE was not proceeding through TLR4 but rather through TLR2 and MyD88. Taken together, our results indicate that LCCWE is a potent activator of NF-κB via TLR2 but not TLR4 and that this activation depends on the MyD88- and TIRAP-dependent signaling pathways.

The molecular mechanisms underlying the coronary arteritis that develops during KD are poorly understood. The animal model of coronary arteritis mimicking KD used in our study was developed by Lehman et al.23,25 A single intraperitoneal injection of sonicated cell walls from group B L casei produced an inflammatory coronary arteritis in mice that in many respects closely resembles coronary arteritis found in children with KD.23,25 Moreover, as in children with KD,9,10 the early coronary arteritis in mice responds to therapy with intravenous immunoglobulin.25,33 Nevertheless, it is important to note that although this mouse model of focal coronary arteritis histopathologically resembles the coronary changes associated with human KD, the potential relevance of our results to humans will require further study in patients with KD.

Several inbred mouse strains exhibit variable susceptibility to LCCWE. For example, strains with defective complement systems and natural killer cell function developed typical or exaggerated coronary arteritis.23,34 Conversely, nude mice developed an attenuated coronary arteritis, suggesting that T cells play a role in the amplification but not the induction of LCCWE-induced coronary arteritis.24 Lehman et al.23,34 previously reported that C3H/HeJ mice do not develop coronary arteritis in response to LCCWE injection, suggesting that TLR4 may potentially be involved in LCCWE-induced coronary arteritis. However, our in vitro and in vivo results do not support a role of TLR4 in LCCWE-induced cellular activation. It should be noted that one of the genetic defects in C3H/HeJ mice corresponds to a missense mutation in the third exon of the gene encoding TLR4 that replaces proline with histidine at position 712 of the polypeptide chain.35 This mutation in the signaling domain of TLR4 renders mice hyporesponsive to LPS.36,37 The most likely explanation to reconcile our present results with those previously reported23,34 is that the mutation in C3H/HeJ involves defects in addition to those that directly affect TLR4 signaling.36,37
The LCCWE mouse model has been used in more recent studies investigating the potential superantigen activity of *L. casei* cell-wall extracts.\(^3\) Brahn et al.\(^3\)\(^2\) showed that an angiogenesis inhibitor suppressed LCCWE-induced coronary vasculitis in mice, suggesting a potential role of endothelial cell proliferation in this model. Recently, Chan et al.\(^3\)\(^8\) reported that the incidence of LCCWE-induced coronary arteritis in IFN-γ-deficient mice was similar to that observed in wild-type mice, demonstrating that IFN-γ is not necessary for induction of arteritis after administration of LCCWE. Superantigens are proteins,\(^2\)\(^9\)\(^3\)\(^0\) but our experiments using proteinase K-digested LCCWE suggest that cellular activation by LCCWE may not require proteins, because MCP-1 secretion by LCCWE-stimulated murine aortic endothelial cells was not affected by pretreatment of the LCCWE preparation with proteinase K. The specific molecular components in LCCWE that induce cellular proinflammatory responses and produce coronary arteritis in mice thus remain unclear but are unlikely to be proteins, and studies to identify and characterize these proinflammatory molecules are currently in progress.

In the past decade, seminal findings have clearly implicated a pivotal role for TLR signaling in host defense, particularly in orchestrating the initial inflammatory response to pathogenic invasion.\(^1\)\(^5\)\(^8\)\(^9\) More recent studies have demonstrated that TLR signaling also has an important function in the pathogenesis of vascular disorders that involve inflammation, notably atherosclerosis and neointimal proliferation after arterial injury.\(^1\)\(^1\)\(^2\)\(^4\)\(^0\)\(^4\)\(^1\) Our present results further extend the general theme emerging from these studies: TLR-mediated signaling pathways transduce signals originating from foreign pathogens or host-derived antigenic molecules that contribute importantly to the pathogenesis of diverse diseases characterized by chronic inflammation and tissue degradation and that have been indirectly linked to infectious agents. Specifically, our results now implicate signaling by TLR2 and the adaptor proteins MyD88 and TIRAP as important contributors to activation of proinflammatory innate immune mechanisms in the LCCWE-induced mouse model of coronary arteritis.

The potential human relevance of these findings will require further study in patients with KD. However, preliminary cDNA microarray analyses suggest that patients with KD show 2- to 13-fold upregulation of expression of TLRs by peripheral blood mononuclear cells, consistent with involvement of TLR signaling in the pathogenesis of the disease.\(^4\) A recent report has emphasized that elevation of such inflammatory markers as C-reactive protein and serum amyloid A are associated with persistence of coronary lesions a decade after the onset of KD, emphasizing the potential clinical significance of chronic activation of innate immune mechanisms in the natural history of the disease.\(^4\)\(^3\) Results reported here add significantly to our understanding of the mechanisms regulating immune activation and localized inflammation in the coronary arteries and may potentially lead to improved treatment to minimize the long-term morbidity and mortality in children with KD.

### Acknowledgments

This work was supported by grants from the National Institutes of Health (HL-66436 and AI-05128 to M.A.).

### References

Kawasaki disease (KD) predominantly strikes children with a characteristic pattern of coronary arteritis and constitutes the leading cause of acquired heart disease among American children. How KD develops remains a mystery, but most consider an unidentified infectious agent to be a contributing or causative factor. Pathogens alert innate immune defenses when molecules unique to microbes (but not produced by the host) interact with 1 of the 11 known mammalian Toll-like receptors (TLRs) that are widely expressed throughout the body. To identify potential mechanisms of KD, we used a mouse animal model of Kawasaki disease.

We hypothesized that the mechanism of LCCWE-induced coronary arteritis may involve TLR signaling and tested this hypothesis by injecting LCCWE into wild-type mice and mice with a specific genetic defect in TLR signaling (LCCWE). We hypothesized that the mechanism of LCCWE-induced coronary arteritis may involve TLR signaling and tested this hypothesis by injecting LCCWE into wild-type mice and mice with a specific genetic defect in TLR signaling.

CLINICAL PERSPECTIVE

Kawasaki disease (KD) predominantly strikes children with a characteristic pattern of coronary arteritis and constitutes the leading cause of acquired heart disease among American children. How KD develops remains a mystery, but most consider an unidentified infectious agent to be a contributing or causative factor. Pathogens alert innate immune defenses when molecules unique to microbes (but not produced by the host) interact with 1 of the 11 known mammalian Toll-like receptors (TLRs) that are widely expressed throughout the body. To identify potential mechanisms of KD, we used a mouse model that recapitulates the histopathological features of KD after injection of Lactobacillus casei cell-wall extract (LCCWE). We hypothesized that the mechanism of LCCWE-induced coronary arteritis may involve TLR signaling and tested this hypothesis by injecting LCCWE into wild-type mice and mice with a specific genetic defect in TLR signaling. LCCWE-induced coronary arteritis in this mouse model required intact TLR2 signaling and the presence of LCCWE but did not depend on TLR4. Our results, together with emerging data that TLRs and MyD88 are among the upregulated genes in monocytes of KD patients compared with controls, suggest the possibility that human KD may also depend on TLR-mediated signaling mechanisms. If this proves to be the case, then more specific and potentially more effective treatments might be devised that target specific aspects of TLR signaling pathways.
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Circulation. 2005;112:2966-2973
doi: 10.1161/CIRCULATIONAHA.105.537530
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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