

# Toll-like Receptor Signaling during Myocardial Ischemia

**A** CUTE myocardial infarction and myocardial ischemia are leading causes of short- and long-term morbidity and mortality in the perioperative period. In part, this relates to the fact that treatment options for a myocardial infarction in surgical patients are extremely limited. For example, anticoagulation or treatment with highly effective inhibitors of platelet aggregation may not be an option due to the risk for bleeding from the operative site. Therefore, it is not surprising that the search for new pharmacologic approaches to treat myocardial ischemia or to render the myocardium more resistant to limited oxygen availability is an area of intense investigation for perioperative scientists.<sup>1–4</sup> In fact, the introduction of intraoperative  $\beta$ -blockers for cardioprotection from ischemia was one of the most significant alterations in anesthesia practice during the past 15 y. In line with the search for novel therapeutic approaches to render the myocardium more resistant to ischemia, a very exciting research study by Wang *et al.*, from the research laboratory of Dr. Wei Chao from the Anesthesia Center for Critical Care Research of the Massachusetts General Hospital in Boston, provides new insight into signaling pathways involving toll-like receptor (TLR)-elicited cardioprotection from ischemia.<sup>1</sup>

TLRs are named after the German word “toll,” which carries the meaning of “great” or “amazing.” In fact, a team of scientists led by Dr. Christiane Nüsslein-Volhard, Ph.D. (Professor, Max Planck Institute, Tübingen, Germany) identified Toll as a gene critical in the embryogenesis of fruit flies by establishing the dorsal-ventral axis of *Drosophila*.<sup>5,6</sup> The name derives from Christiane Nüsslein-Volhard’s 1985 exclamation, “Das war ja toll,” which translates as “that’s amazing,” in reference to the underdeveloped ventral portion of a fruit fly larva. Together with Edward B. Lewis and Eric Wieschaus, she received the Nobel Prize in Physiology or Medicine in 1995 for her discoveries concerning the genetic control of early embryonic development. Ten years after the original discovery of the Toll gene, a novel role for Toll beyond its function in embryogenesis was discovered.<sup>7</sup> In fact, mutant flies deficient in Toll were more susceptible to fungal or bacterial infections.<sup>7</sup> In 1997, a group of scientists from Yale University led by Ruslan Medzhitov (Professor, Yale University School of Medicine, New Haven, CT) and Charles Janeway Jr. (Professor, Yale University School of Medicine, New Haven, CT) (1943–2003) described a human homolog of the *Drosophila* Toll protein that had the resemblance of a cytoplasmic receptor (“Toll-like”).<sup>8</sup> In this landmark study, human Toll was identified as a type I

transmembrane protein with an extracellular domain consisting of a leucine-rich repeat domain, and a cytoplasmic domain homologous to the cytoplasmic domain of the human interleukin (IL)-1 receptor. Moreover, constitutively active mutant of human Toll transfected into human cell lines could induce the activation of nuclear factor (NF) $\kappa$ B and the expression of NF $\kappa$ B-target genes.<sup>8</sup> As such, today’s view of “Toll-like receptors” has evolved toward a class of receptor proteins that play a key role in the innate immune system.<sup>9</sup> They are single-membrane-spanning, noncatalytic receptors that recognize structurally conserved molecules derived from microbes (pattern recognition receptors). For example, studies of mice deficient in TLR4—the key TLR for the recognition of lipopolysaccharide—show hyporesponsiveness to lipopolysaccharide treatment.<sup>10</sup> Currently, 10 TLR family members have been described in human.<sup>11</sup> They differ in their specificity for different microbial compounds. For example TLR2 can be activated by lipoteichoic acid from Gram-positive bacteria, TLR4 by lipopolysaccharide from Gram-negative bacteria, or TLR5 by flagellin, the filament of bacterial flagella found on most motile bacteria.<sup>12,13</sup> Activation of TLRs involves specific signal transduction pathways, including myeloid differentiation factor 88 (MyD88)- or TIR-domain-containing adaptor protein-inducing interferon- $\beta$ -mediated transcription factor (Trif)-dependent (MyD88-independent) pathways that culminate in the activation of NF $\kappa$ B, thus promoting a pro-inflammatory host program.<sup>11,14</sup> TLR signaling has been implicated in many forms of inflammatory disorders in human. For instance, a cosegregating missense mutation (Asp299Gly and Thr399Ile) affecting the extracellular domain of the TLR4 receptor is associated with a blunted response to inhaled lipopolysaccharide in humans.<sup>15</sup> This common Asp299Gly polymorphism of TLR4 also predicts low concentrations of circulating inflammatory mediators and confers an increased risk of severe infections but carries a reduced risk of atherosclerosis in human.<sup>16</sup>

Although TLR signaling has been extensively studied in the context of innate immunity, other studies have also implicated TLR signaling in noninfectious tissue injury, such as occurs in the context of myocardial infarction. As such, the studies by Wang *et al.*<sup>1</sup> build on the observation that previous administration of a small, nonlethal dose of the TLR4 agonist lipopolysaccharide results in robust cardioprotection from subsequent myocardial ischemia–reperfusion injury.<sup>17</sup> To gain mechanistic insight on how TLR4 activation confers cardioprotection from

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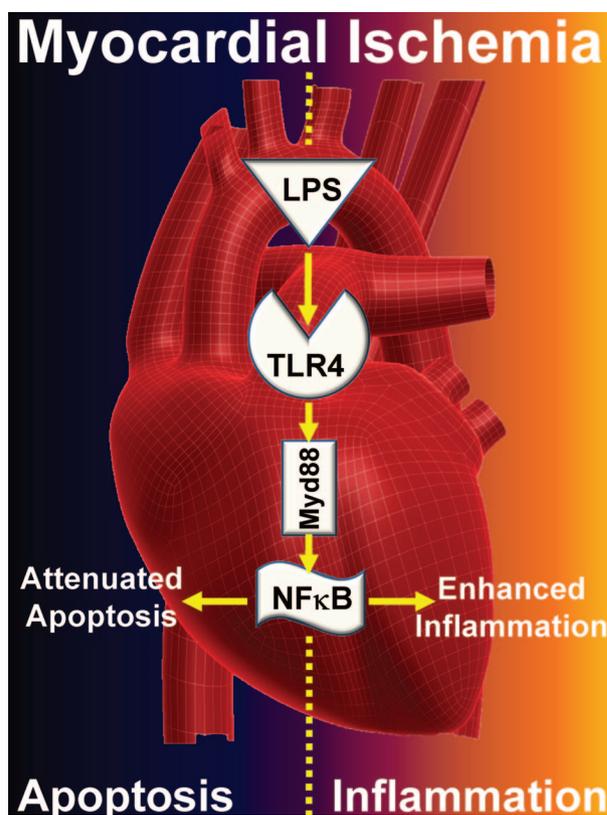
◆ This Editorial View accompanies the following article: Wang E, Feng Y, Zhang M, Zou L, Li Y, Buys ES, Huang P, Brouckaert P, Chao W: Toll-like receptor 4 signaling confers cardiac protection against ischemic injury via inducible nitric oxide synthase- and soluble guanylate cyclase-dependent mechanisms. ANESTHESIOLOGY 2011; 114:603–13.

ischemia, the team of scientists combined very elegant pharmacologic and genetic approaches in mice treated with systemic lipopolysaccharide. For this purpose, lipopolysaccharide- or vehicle-treated mice were subsequently (24 h later) exposed to myocardial ischemia using a Langendorff apparatus, and myocardial infarct sizes and cardiac function were examined. These studies demonstrated robust cardioprotection with TLR4-agonist treatment (approximately 50% reduction in infarct size) that was TLR4 specific. In fact, *Tlr4*<sup>-/-</sup> mice were not protected by lipopolysaccharide pretreatment, whereas *Tlr2*<sup>-/-</sup> mice demonstrated similar cardioprotection as wild-type animals. Studies in mice deficient for MyD88 or Trif revealed MyD88-dependent and Trif-independent signal transduction. Consistent with previous *in vitro* studies from the laboratory of Dr. Chao,<sup>18</sup> their current studies provide genetic *in vivo* evidence that these signaling events finally converge on inducible nitric oxide synthase and soluble guanylate cyclase. The authors have to be congratulated on putting together such a comprehensive number of genetic models that allowed their team to convincingly delineate this pathway *in vivo*. In contrast to studies on anesthetic preconditioning, where an *in vivo* knockdown of the receptor mediating the specific effects of a “cardioprotective anesthetic” has been literally impossible (currently, an “isoflurane-receptor knockout mouse” does not exist), the authors were able to target specifically the receptor critical for TLR-dependent cardioprotection and provide an extremely convincing level of evidence in support of their hypothesis *via* a combination of genetic and pharmacologic approaches.

In contrast to their findings, and somewhat paradoxical to the central hypothesis of their article, are studies in TLR-deficient mice exposed directly to myocardial ischemia–reperfusion injury. For example, an *in situ* study of myocardial ischemia–reperfusion injury exposed gene-targeted mice for *Tlr4* to 60 min of coronary artery ligation, followed by 24 h of reperfusion. The authors observed attenuated infarct sizes in *Tlr4*-deficient mice in conjunction with attenuated myocardial inflammation, as assessed by neutrophil accumulation, lipid peroxidase, and complement deposition.<sup>19</sup> Similar to these apparent discrepancies, previous studies have found a dual role of signaling pathways that result in activation of NFκB during ischemia-induced inflammation. In fact, a very elegant study from the laboratory of Michael Karin investigated the role of NFκB in acute inflammation caused by intestinal ischemia–reperfusion through selective ablation of IκB kinase (IKK)-β, the catalytic subunit of IKK that is essential for NFκB activation.<sup>20</sup> Ablation of IKK-β in enterocytes prevented the systemic inflammatory response that culminates in multiple organ dysfunction syndrome and is normally triggered by gut ischemia–reperfusion. However, IKK-β removal from enterocytes also resulted in severe apoptotic damage to the reperfused intestinal mucosa. Thus, these studies demonstrate a dual function of the NFκB system, which is responsible for both tissue protection and systemic inflammation.<sup>20</sup> Consistent with these findings, a pro-inflammatory imbalance after myocardial ischemia–reperfusion injury is attenuated in *Tlr4*<sup>-/-</sup> mice,<sup>19</sup> whereas TLR-agonist treatment

is associated with cardioprotective responses *via* dampening ischemia-driven cardiomyocyte apoptosis (fig. 1).<sup>17</sup>

Ultimately the goal of the study by Wang *et al.* is to identify novel therapeutic approaches for perioperative myocardial injury,<sup>1</sup> but some future challenges have to be overcome. The current studies were carried out in a Langendorff apparatus in the absence of inflammatory cells. It is conceivable that activation of myocardial TLRs and subsequent activation of an NFκB-elicited pro-inflammatory program could enhance inflammatory cell trafficking into the myocardium. As such, it will be an important step to test TLR4 agonists in intact models of myocardial ischemia–reperfusion injury in the presence of inflammatory cells.<sup>21,22</sup> Although other studies seek to identify ways to dampen hypoxia-elicited inflammation,<sup>23–26</sup> the consequences of a single



**Fig. 1.** Dual role of Toll-like receptor signaling in myocardial ischemia–reperfusion injury. A study by Wang *et al.* published in the current edition of ANESTHESIOLOGY provides genetic and pharmacologic evidence for a cardioprotective role of Toll-like receptor signaling (TLR) during myocardial ischemia. Pretreatment with lipopolysaccharide (LPS) provides potent protection from subsequent myocardial ischemia, involving signal transduction through MyD88, and convergence on inducible nitric oxide synthase and guanylate cyclase. As such, TLR4-dependent activation of nuclear factor (NF)κB could provide cardioprotection from ischemia by dampening programmed myocardial cell death (apoptosis). However, previous studies in knockout mice for TLR4 or MyD88 demonstrate attenuated susceptibility to myocardial ischemia–reperfusion injury. These later findings could reflect increases in myocardial inflammation during ischemia–reperfusion injury.

bolus treatment with lipopolysaccharide on myocardial inflammation after ischemia reperfusion injury needs to be clearly addressed. In addition, the therapeutic window for TLR agonist treatment has yet to be defined. The authors can produce robust cardioprotection with a 24-h pretreatment approach, but it would be highly desirable for the perioperative setting to use a TLR agonist at the onset of myocardial ischemia. As pointed out by the authors, additional limitations of current TLR4 agonists are their pyrogenic side effects. As such, the development of nonpyrogenic TLR agonists will be required to directly target this pathway for the perioperative treatment of myocardial ischemia reperfusion injury. However, none of these comments should distract from the enthusiasm about the studies provided by the laboratory of Dr. Chao. In our mind, the elegant experimental design, the approach of combining pharmacologic and genetic studies, the vision of characterizing molecular targets and pathways beyond the field of anesthetics, and the important therapeutic implications of their research work set the stage for future innovations in perioperative cardioprotection. No doubt, Dr. Chao and his colleagues have set the bar very high.

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