Coronary heart disease is an inflammatory disorder of the coronary vessels. With a prevalence of approximately 20% in the general population, it is an endemic problem in industrialized countries. It has been the leading cause of death in the past 80 years or so in the United States. Coronary heart disease may lead to acute coronary syndromes. These cover a wide spectrum of clinical presentations including unstable angina, non–ST-segment elevation myocardial infarction, ST-segment elevation myocardial infarction, and sudden cardiac death. The common anatomic substrate of acute coronary syndromes is coronary thrombosis or rupture of an atherosclerotic plaque leading to an obstruction of the vessel and subsequent ischemia in the corresponding myocardial area. Thus, prompt reperfusion by primary percutaneous coronary intervention or fibrinolysis combined with early anti-ischemic therapy together with analgesic agents, to restore blood flow and diminish myocardial necrosis associated with prolonged ischemia.

A Brief History of TLRs

These evolutionary, highly conserved molecules were first discovered in 1985 as developmental regulators of dorsal-ventral polarity in Drosophila.5 Experiments by Christine Nüsslein-Volhard suggested that drosophila Toll (dToll) plays an important role in the morphogenesis of animals. When the toll gene is mutated in female Drosophila, they are only able to produce dorsalized embryos, in which all embryonic cells behave like the dorsal cells of the wild-type embryo.

It had been shown previously that antimicrobial peptides play a major role in Drosophila immunity.6 A few years later, Bruno Lemaître, in the laboratory of Jules Hoffmann, was able to show that a defect in dToll in adult Drosophila leads to a profound defect in the ability to fight fungal infections because the antimicrobial peptide drosomycin could not be produced.7 Interestingly, the induction of a host response was dependent on the activation of the transcription factor Dorsal, a homologue of nuclear factor-kB/Rel.8,7 This linked dToll to immune mechanisms for the first time. Additionally, Nicholas Gay and Fiona Keith found that the cytosolic signaling molecules exist.10,11 This common ancestry suggested that this domain, now known as the Toll/IL-1RI homologue domain, is highly important for innate immunity. It also suggested that this domain, now known as the Toll-interleukin receptor (TIR) domain, was involved in signaling processes not only in the restricted context of insect development but also in the generation of initial responses to infection in other species, including humans. Now, researchers had enough information to search for Toll-like molecules in humans. Charles A. Janeway and Ruslan Medzhitov set out to solve what Janeway coined the “immunologist’s dirty little secret”: what controlled the expression of costimulatory molecules on dendritic cells? In 1997, Janeway and Medzhitov published the cloning of a human homologue of the Drosophila Toll protein, which clearly induced the costimulatory molecules CD80 and CD86. This protein was later renamed TLR-4 for (human) TLR4.8 Unfortunately, Charles A. Janeway died in 2003 at only 60 years old.
The ligand for hTLR4 was still unknown. Earlier, Bruce Beutler\textsuperscript{13} had purified tumor-necrosis factor (TNF-\(\alpha\)) and thus knew that the production of TNF-\(\alpha\) is upregulated on stimulation of macrophages by lipopolysaccharide (LPS), a structural part of the cell wall of Gram-negative bacteria. However, at that time he did not know what the receptor for LPS was. Beutler’s group\textsuperscript{14} intensively studied an LPS-nonresponsive mouse strain, C3H/HeJ, and revealed by positional cloning analysis that a point mutation in the TIR domain of TLR4 was responsible for the defect in LPS signal transduction and that LPS in fact is the ligand for TLR4.

Jules Hoffmann and Bruce Beutler share half of the 2011 Nobel Prize in Physiology or Medicine for their contributions to innate immune recognition. The innate-adaptive connection of Ruslan Medzhitov and the late Charles A. Janeway is now a fundamental principle in immunology, and their report published in 1997 certainly is one of the greatest contributions to immunology.\textsuperscript{8}

**TLRs as Therapeutic Target in I/R Injury**

The first years of the new millennium, shortly after the discoveries mentioned above, saw a boom for TLR research with new exciting discoveries on a frequent basis. Soon, data were available that showed an effect of mutations in TLRs or subsequent signaling cascades on physiological processes. The interest in TLRs and TLR signaling was such that most, if not all, immunologic phenomena were illuminated from a TLR perspective.

In the year 2000, data suggested that common cosegregating missense single nucleotide polymorphisms Asp299Gly and Thr399Ile affecting the extracellular domain of the TLR4 receptor are associated with a blunted response to inhaled LPS in humans.\textsuperscript{15}

The observation that mutations in TLR signaling lead to altered physiology or pathophysiology and the fact that TLRs are deeply involved in many (immunologic) processes excited researchers, including us, and suggests that TLR signaling can be therapeutically modulated to interfere with pathological processes in different disease entities.

Although many questions about TLRs are unsolved, it is now accepted that the 10 TLRs identified in humans are leucin-rich repeat receptors that act as pattern recognition receptors. They signal via 4 adapter proteins—MyD88, MAL, TRIF, and TRAM—and primarily the transcription factor nuclear factor-\(\kappa\)B to initiate innate and instruct adaptive immunity. It also became clear that not only “pathogen-associated molecular patterns” (PAMPs) are ligands for TLRs but also endogenous molecules such as hyaluronic acid (TLR2 signaling summarized in Figure 1). These endogenous molecules were termed “danger-associated molecular patterns” (DAMPs). DAMPs are assumed to be key drivers of “sterile” inflammation. These molecules, which are breakdown products of the extracellular matrix, products of proteolytic cascades activated by I/R injury and released from necrotic cells, alarm the immune system to promote tissue repair and to prevent infection of damaged tissue by ubiquitously present pathogens. Several endogenous molecules have been proposed to bind TLRs as DAMPs: necrotic or dying cells, HMGB1, S100 calcium-binding proteins, cardiac myosin, serum amyloid A, fibrinogen, heat shock proteins, and amyloid\(\beta\), are released from injured tissue, for example, after ischemia/reperfusion (I/R) injury, bind to TLRs. Signaling downstream of TLRs is likely to be initiated by homodimerization (eg, TLR2–TLR2) or heterodimerization (TLR1–TLR2 and TLR2–TLR6). This causes the intracellular signaling domains of the receptors to dimerize. Myeloid differentiation primary response protein 88 (MyD88) is a universal adaptor for TLRs, with the exception of TLR3. MyD88 adaptor-like protein (MAL) is needed to bridge the recruitment of MyD88 to TLR2 and TLR4. After its recruitment, signal transduction ensues through the recruitment of 2 interleukin (IL)-1 receptor–associated kinases (IRAKs), IRAK4 and IRAK1. This recruitment, in turn, leads to activation of tumor necrosis factor (TNF) receptor–associated factor (TRAF)6 and, subsequently, the activation of nuclear factor (NF)-\(\kappa\)B, which leads to transcription of proinflammatory cytokines, for example, TNF-\(\alpha\). The following inflammation might propagate tissue injury. The \(\alpha\)-TLR2 antibody OPN-305 inhibits TLR2-induced signaling and ameliorates the sequelae of I/R injury in a pig model of myocardial infarction.

**Figure 1.** Toll-like receptor (TLR)2 signaling: Danger-associated molecular patterns (DAMPs), such as necrotic or dying cells or their parts, HMGB1, S100 calcium-binding proteins, cardiac myosin, serum amyloid A, fibrinogen, heat shock proteins, and amyloids, are released from injured tissue, for example, after ischemia/reperfusion (I/R) injury, bind to TLRs. Signaling downstream of TLRs is likely to be initiated by homodimerization (eg, TLR2–TLR2) or heterodimerization (TLR1–TLR2 and TLR2–TLR6). This causes the intracellular signaling domains of the receptors to dimerize. Myeloid differentiation primary response protein 88 (MyD88) is a universal adaptor for TLRs, with the exception of TLR3. MyD88 adaptor-like protein (MAL) is needed to bridge the recruitment of MyD88 to TLR2 and TLR4. After its recruitment, signal transduction ensues through the recruitment of 2 interleukin (IL)-1 receptor–associated kinases (IRAKs), IRAK4 and IRAK1. This recruitment, in turn, leads to activation of tumor necrosis factor (TNF) receptor–associated factor (TRAF)6 and, subsequently, the activation of nuclear factor (NF)-\(\kappa\)B, which leads to transcription of proinflammatory cytokines, for example, TNF-\(\alpha\). The following inflammation might propagate tissue injury. The \(\alpha\)-TLR2 antibody OPN-305 inhibits TLR2-induced signaling and ameliorates the sequelae of I/R injury in a pig model of myocardial infarction.
severe sepsis but showed a reduction of infarct area by this intervention. The results were tainted a bit by the fact that Eritoran was given 10 minutes before ischemia.

There is accumulating evidence for an important role of TLR2 in I/R injury, and similar mechanisms as in TLR4-mediated I/R injury have been found. TLR2 is very versatile and binds more ligands than any other member of the TLR family. The heterodimerization of TLR2 with either TLR1 or TLR6 is essential for the recognition of bacterial lipopeptides and lipoproteins, such as the synthetic triacylated Pam3CSK4, lipoteichoic acid (LTA), and MALP-2, a ligand from *Mycoplasma spp*. Diacylation of lipopeptides is required for TLR2 and TLR6 signaling, and triacylation of lipopeptides is required for TLR1 and TLR2. We can currently only speculate regarding other endogenous ligands that are liberated in I/R injury and are recognized by TLR2 heterodimers.

Selejan and colleagues found an upregulation of TLR2-positive monocytes and inflammatory cytokines in patients with myocardial infarction and even more in cardiogenic shock compared with healthy control subjects. This, in the context of previous work, may suggest that TLR2-positive monocytes are recruited to the heart, where they do deleterious work.

There is also “the other side” of TLR2 inhibition in I/R injury: TLR2-deficient mice had no benefit from being TLR2 deficient in a model of left anterior descending artery occlusion and showed inadequate remodeling and marked left ventricular dilation. In the same report, though, the authors confirmed data from the group of Arslan that had demonstrated a beneficial effect of a blocking TLR2 antibody (OPN-301) in mice. Importantly, the authors reported that infarct size is determined by circulating TLR2 expression, adding to the observation of Selejan and others that TLR2 expression in circulating leukocytes is related to infarct size.

Overall, this suggests that if TLR2 inhibition exerts its effect too long on the damaged myocardium, wound healing might be impaired, thus resulting in unfavorable remodeling of the ventricle. As often with the immune system, a dysregulated, unproportional reaction is detrimental. This has been illustrated by the work of Khor and colleagues, who showed that heterozygosity for a single nucleotide polymorphism of the adapter protein MAL protects from invasive pneumococcal disease, bacteremia, malaria, and tuberculosis. This probably is because 1 copy of MAL is inactive and thus immunity appears to be “tempered.” Also in I/R injury, inflammation can be a double-edged sword: one edge is the influx of inflammatory cells that contributes to infarct size and has to be blocked; the other edge is wound healing, which is undoubtedly an important aspect of inflammation. Timing and dose of a TLR2-blocking antibody probably will be important in clinical trials.

Fatih Arslan and colleagues have now tested a humanized anti-TLR2 antibody of clinical grade in a model of left circumflex artery occlusion.

Administration of OPN-305 after 60 minutes of ischemia was followed by an additional 15 minutes of ischemia and 24 hours of reperfusion. All animals received amiodarone, clopidogrel, and acetylsalicylic acid in preparation for ischemia; 3 groups (n = 10 per group) were treated with OPN-305 at different doses, and a fourth group (n = 10) received vehicle.

Despite the optimal pretreatment of the animals by using clopidogrel and acetylsalicylic acid to inhibit platelet aggregation, OPN-305 dose-dependently still reduced infarct size by almost 50% in the highest dose. OPN-305 achieved this after 60 minutes of ischemia, 15 minutes before reperfusion. This can be regarded as a realistic situation in patients with ST-segment elevation myocardial infarction. It is also a situation that can be covered in a clinical trial if OPN-305 can be given preclinically or directly in the emergency room before target vessel revascularization.

The study by Arslan could not explain the observed effects on a mechanistic basis. The effects probably are exerted not by inhibition of parenchymal TLR2 but by inhibition of TLR2 on circulating cells that are recruited to a lesser extent to the injured myocardium. Additionally, as discussed by the authors, a second effect might be that TLR2 inhibition reduces platelet clotting (Figure 2). This effect might well add to the efficacy of OPN-305 in acute coronary syndromes, but this probably is not the whole story of OPN-305, because animals were pretreated with clopidogrel and salicylic acid and yet infarct size was greatly reduced. Whereas mechanistic explanations are needed to understand how the disease works...
Disclosures

None.

References


32. Lepper and Bals Blocking TLR-2 in I/R Injury

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On the Edge: Targeting Toll-Like Receptor 2 in Ischemia/Reperfusion Injury
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