Therapeutic Targeting of Toll-Like Receptors for Infectious and Inflammatory Diseases and Cancer

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Abstract

Since first being described in the fruit fly Drosophila melanogaster, Toll-like receptors (TLRs) have proven to be of great interest to immunologists and investigators interested in the molecular basis to inflammation. They recognize pathogen-derived factors and also products of inflamed tissue, and trigger signaling pathways that lead to activation of transcription factors such as nuclear factor-κB and the interferon regulatory factors. These in turn lead to induction of immune and inflammatory genes, including such important cytokines as tumor necrosis factor-α and type I interferon. Much evidence points to a role for TLRs in immune and inflammatory diseases and increasingly in cancer. Examples include clear roles for TLR4 in sepsis, rheumatoid arthritis, ischemia/reperfusion injury, and allergy. TLR2 has been implicated in similar pathologic conditions and also in systemic lupus erythematosus (SLE) and tumor metastasis. TLR7 has also been shown to be important in SLE. TLR5 has been shown to be radioprotective. Recent advances in our understanding of signaling pathways activated by TLRs, structural insights into TLRs bound to their ligands and antagonists, and approaches to inhibit TLRs (including antibodies, peptides, and small molecules) are providing possible

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I. Introduction

The discovery of Toll-like receptors (TLRs) heralded the renaissance of interest in innate immunity for immunologists, who, despite extensive studies having been carried out in the area (mainly by those investigators interested in inflammation), previously thought it to be relatively crude, nonspecific, and somewhat unpromising for specific therapeutic targets for infectious and inflammatory diseases. Extensive analysis of TLRs, however, has revealed specificity in terms of ligand recognition, expression in different cell types and tissues, and, importantly, a role for TLRs in the pathogenesis of multiple diseases involving both the innate and adaptive immune systems. There are 10 TLRs in humans and they recognize different microbial ligands during infection (O’Neill and Bowie, 2007). There is also a growing body of evidence to indicate that certain TLRs also sense products of damaged tissue. Both pathogen-derived factors and also damaged tissue will provoke inflammation; it has therefore been hypothesized that TLRs initiate the inflammatory response in both cases. Also of interest are the different signaling pathways activated by TLRs. Five different adapter proteins are recruited in different combinations to different TLRs, allowing for tailored responses to each pathogen (O’Neill and Bowie, 2007). There are several protein kinases downstream of these adapters, notably the IL-1 receptor-associated kinase (IRAK) family and TIR-1. These activate pathways leading to the activation of the respective transcription factors nuclear factor κB (NFκB) and interferon regulatory factor 3 (IRF3), which in turn induce various immune and inflammatory genes. The human TLRs, their ligands, and the signals they activate are shown in Fig. 1.

Studies on TLR-deficient mice have implicated them in multiple pathologic conditions, and the targeting of either the TLRs themselves or the signals they generate is proving to be of great interest. There is sufficient validation around certain TLRs, as we describe in this review, to justify them as therapeutic targets. These validation criteria are standard—expression in disease; activation leading to enhanced disease in models; protection of TLR-deficient mice against disease; and provision of risk factors for disease by single nucleotide polymorphisms in TLRs or their adapters. Finally, a key output from TLRs are inflammatory cytokines such as TNF and IL-6, which have proven to be excellent targets for inflammatory diseases such as rheumatoid arthritis. Targeting TLRs will therefore in all likelihood prevent the induction of many immune and inflammatory proteins. The wide tissue distribution of TLRs, however, may make it difficult to determine whether an agonist or an antagonist will be most effective therapeutically. In this review, we systematically describe and discuss the potential role of each TLR in disease and speculate on the prospect of future targeting of TLRs. The hope is that given the level of validation around them, they should prove to be a very interesting new class of targets for diseases where there is still an unmet medical need.

II. Toll-Like Receptor 4: Agonism and Antagonism

TLR4 was the first TLR identified (Medzhitov et al., 1997) and was characterized as a pattern recognition receptor through the study of the lipopolysaccharide (LPS)-resistant C3H/HeJ and C57BL/10ScCr mice strains. Mapping and sequencing identified the Tlr4 gene as a candidate site for the mutation causing LPS resistance. In C3H/HeJ mice, the Tlr4 gene has a single A→C point mutation, resulting in a P712H substitution in the TIR domain of TLR4 (Poltorak et al., 1998; Qureshi et al., 1999) conferring dominant-negative activity on TLR4 in these mice (Vogel et al., 1999). The C57BL/10ScCr strain is homozygous for a null mutation of Tlr4 (Poltorak et al., 1998). The role of TLR4 in LPS signaling was confirmed in Tlr4(−/−) mice that were hyporesponsive to LPS (Hoshino et al., 1999). Mutations in the human Tlr4 gene, corresponding to D299G and T399I, were shown to associate with hyporesponsiveness to inhaled LPS (Arbour et al., 2000), and expression of these mutants in vitro shows reduced activation in response to LPS (Rallabhandi et al., 2008). Expression of TLR4 alone does not confer responsiveness of cells to LPS. TLR4 was found to require an additional protein, MD-2, with which it has to be associated to be activated by LPS (Shimazu et al., 1999), and mice lacking MD-2 do
not respond to LPS (Nagai et al., 2002). A number of MD-2 polymorphisms have been identified that alter LPS binding and/or activation (Hamann et al., 2004; Gu et al., 2007; Vasl et al., 2008). LPS interaction with MD-2/TLR4 involves at least two other proteins. LPS binds first to lipopolysaccharide binding protein in serum (Schumann et al., 1990) and is then transferred to CD14 (Wright et al., 1990). The major role for CD14 is to enhance the sensitivity of the MD-2/TLR4 signaling complex, dropping the binding affinity for LPS to picomolar concentrations (Gioannini et al., 2004). Mice without CD14 are resistant to endotoxic shock (Haziot et al., 1996).

A. Ligand Recognition at Myeloid Differentiation Factor 2/Toll-Like Receptor 4 Protein Complex: A Basis for Antagonism?

The best characterized ligand for the MD-2/TLR4 complex is lipid A (the biologically active component of LPS). The lipid A domain of LPS consists of a disaccharide to which various substituents, including acyl chains of variable length and number, are attached (Raetz and Whitfield, 2002). *Escherichia coli* lipid A is usually hexa-acylated, whereas a tetra-acylated lipid A, lipid IVa, is also produced by *E. coli* as an intermediate in the lipid A biosynthetic pathway (Raetz and Whitfield, 2002). Different lipid A structures may be agonists or antagonists at the MD-2/TLR4 (Walsh et al., 2008). Subtle alterations in lipid A structure profoundly alter its biological activity, such that a synthetic compound CRX-527 is an agonist, but decreasing the secondary acyl chain length below 6 or increasing it above 14 results in a loss of agonist activity (Stöver et al., 2004).

Binding of lipid A to MD-2/TLR4 (Raetz et al., 2006) induces structural rearrangements that trigger oligomerisation of TLR4 and initiate signal transduction (Re and Strominger, 2002, 2003; Visintin et al., 2003; Gangev and Gay, 2004; Viriyakosol et al., 2006). MD-2 binds to lipid A (Viriyakosol et al., 2001) and was therefore thought to be the key player in lipid A recognition, whereas TLR4, unlike other TLRs, was not thought not to participate directly in lipid A binding (Viriyakosol et
Lipid A is recognized by MD-2 after transfer from CD14, which does not participate in the signaling complex (Gioannini et al., 2004). The first ligand bound structures for MD-2 (Ohto et al., 2007) and TLR4/MD-2 (Kim et al., 2007) were both complexes bound to antagonists. These studies led to the hypothesis that lipid A induces MD-2 to change shape, which would result in a change in conformation of TLR4 to trigger signaling. Very recently, lipid A in complex with MD-2 was crystallized, however, and these data show that MD-2 does not change shape when bound to an agonist (Park et al., 2009). The structure of the TLR4/MD-2 antagonist-bound complex is shown in Fig. 2.

The first crystal structure for human MD-2 is of the protein bound to lipid IVa (an antagonist at human MD-2/TLR4). In this structure, the four acyl chains of lipid IVa fills the deep hydrophobic cavity formed by the two β sheets in MD-2. The phosphorylated glucosamine backbone is located at the entrance to the hydrophobic cavity (Ohto et al., 2007). In the MD-2/TLR4 complex, MD-2 is complexed to another antagonist, eritoran. Similar to the MD-2-lipid IVa structure, the four acyl chains of Eritoran occupy approximately 90% of the solvent-accessible volume of the pocket. Two of the acyl chains are fully extended conformation within the binding pocket, but two of the acyl chains are bent in the middle. The di-glucosamine backbone of Eritoran, like the diglucosamine backbone of lipid IVa, is fully exposed to solvent (Kim et al., 2007). What happens to the extra acyl chains in lipid A structures that have more than 4 acyl chains, such as hexaacylated lipid A? Do the extra acyl chains somehow associate with TLR4?

To answer these questions many mutagenesis, structural modeling and crystallization studies have been performed. There was controversy as to whether TLR4 participates directly in ligand binding and discrimination. TLR4 could play a secondary role in ligand binding, as residues in MD-2 (C95 and C105) important for TLR4 binding (Mullen et al., 2003; Re and Strominger, 2003), are located at the rim of the ligand-binding cavity (Ohto et al., 2001).

FIG. 2. The structure of TLR4/MD-2: molecular basis for ligand binding. A, the structure of human TLR4 (turquoise) bound to MD-2 (yellow) is taken from the crystal structure (Kim et al., 2007). The single nucleotide polymorphisms in TLR4 (D299G and T399I) are shown in green, the cysteine residues in MD-2 critical for LPS binding (Cys95 and Cys105) are shown in red, and the residues in MD-2 (Phe126 and His155) critical for receptor dimerization in response to LPS are shown in pink. B, a model to suggest the structural basis of ligand activation of TLR4/MD-2 (lateral and top views). Using the structural data, a model was made to explain how TLR4/MD-2 might dimerize to form an active complex (Walsh et al., 2008). The two TLR4 molecules are represented in purple and turquoise and the two MD-2 molecules in yellow and green. In this model, there are contacts between the two TLR4 proteins, and each MD-2 touches both TLR4 proteins (see the top view). TLR4 SNP D299G is indicated in red and T399I is indicated in black.
This was supported by the higher LPS affinity of the MD-2/TLR4 complex (with a Kd of around 3 nM (Akashi et al., 2003)) compared with MD-2 (Kd of 65 nM (Viriyakosol et al., 2001)) on its own. MD-2 binds to TLR4 on a lateral surface of the TLR4 solenoid near to the N terminus (Kim et al., 2007). One approach to understand how active MD-2/TLR4 complexes are formed is to exploit the marked mammalian species differences in the activity of different types of lipid A that behave as agonists or antagonists at the MD-2/TLR4 complex (Akashi et al., 2001; Kawasaki et al., 2001; Muroi and Tanamoto, 2006). Lipid IVa is an agonist in the mouse, a partial agonist in the horse and is an antagonist for human cells (Akashi et al., 2001; Sauter et al., 2007). Using chimeric constructs made from human and horse TLR4 and MD-2, sequences have been identified in both proteins that are required for lipid IVa to signal. A molecular model using this data predicted that interchain contacts occur between MD-2 and TLR4 and explain why two highly conserved residues in MD-2 (F126 and H155) are critical for receptor dimerization in response to LPS (Kobayashi et al., 2006) by contributing to TLR4 cross-linking. The model also predicted the presence of TLR4 receptor-receptor contacts. The assembly of the active TLR4 complexes was predicted to be a stepwise process, with initial MD-2/TLR4 contacts induced by binding of lipid A promoting the subsequent homodimerization of the receptor ectodomains (Walsh et al., 2008). Crystallization of LPS bound to MD-2/TLR4 showed that the predictions of the mutagenesis data and the modeling were remarkably accurate. This fascinating structure shows the main dimerization face of TLR4 being between leucine-rich repeats 15–17. It also shows that five of the LPS acyl chains are fully accommodated in MD-2, the sixth acyl chain is exposed to interact with TLR4, and the LPS phosphate groups interact with the positively charged residues in TLR4 (Park et al., 2009). Several questions remain unanswered, however, including how the TLR4 single-nucleotide polymorphisms (D299G and T399I) in human TLR4, which are not in the N-terminal MD-2 binding site or in the dimerization interface on TLR4, reduce lipid A responsiveness (Arbour et al., 2000; Rallabhandi et al., 2006). The mechanism for this is unclear, but the mutations could either affect the cooperative binding of lipid A or alter the conformational changes that occur during ligand-induced signal transduction.

The interaction of lipid A with the MD-2/TLR4 complex is increasingly well understood, but a number of ligands other than lipid A have been identified as TLR4 agonists. These include endogenous ligands [such as high mobility group box 1 protein (HMGB1), 60-kDa heat shock protein (HSP60), HSP70, type III repeat extra domain of fibronectin (EDA), hyaluronic acid oligosaccharides, heparin sulfate polysaccharide fragments, and fibrinogen], other pathogen-derived ligands (such as Streptococcus pneumoniae HSP60, mouse mammary tumor virus envelope proteins, and respiratory syncytial virus fusion protein), and plant ligands (paclitaxel) (Gay and Gangoth, 2007). The molecular nature of how these ligands interact with TLR4 and whether MD-2 is required is not well understood. It does seem that EDA (Okamura et al., 2001), C. pneumoniae HSP60, and respiratory syncytial virus fusion protein (Rallabhandi et al., 2006) do require MD-2 for activation of TLR4 although the molecular basis for this is unclear. Some ligands, such as oxidized low-density lipoprotein and β-amyloid, may induce heterotrimeric complexes of CD36/TLR4/TLR6 (Stewart et al., 2008), but whether the formation of these type of complexes is common for TLR4 remains to be clarified. Until crystallographic evidence shows that these protein ligands bind to the receptor to induce a conformational change and activate signaling, it remains controversial as to whether these proteins are true ligands for TLR4.

B. Efficacy at Toll-Like Receptor 4: Recruitment of Adapter Proteins

Efficacy at TLR4 induced by ligand binding involves dimerization or oligomerization of receptor chains (Saitoh et al., 2004). This in turn probably causes protein conformational changes in the receptor, resulting in the association of two receptor TIR domains (Gay et al., 2006). Fluorescence resonance energy transfer microcopy showed that the TLR9 TIR domains undergo a large positional change on ligand binding (Lorenz et al., 2002); therefore, it is reasonable to assume that this may occur with other TLRs on dimerization. The association of the receptor TIR domains would provide a new scaffold that allows the recruitment of specific adapter proteins to form a postreceptor signaling complex. Five adapter proteins function in TLR signaling, and they all have TIR domains (O’Neill and Bowie, 2007). Activated TLR4 recruits two distinct adapter protein pairs, Mal/MyD88 and TRAM/TRIF. These molecules are thought to engage directly with the receptor and to act as “bridging adapters” for the recruitment of MyD88 and TRIF, respectively. Mal is required for rapid activation of the NFκB transcription factor and the production of proinflammatory cytokines such as TNFα. TRAM stimulates sustained NFκB activation and a different signaling pathway, leading to activation of IRF3. IRF3 induces expression of a set of genes distinct from that of NFκB, such as IFNβ and the chemokine RANTES (regulated on activation normal T cell expressed and secreted) (O’Neill and Bowie, 2007).

Mutagenesis and molecular modeling studies suggest that ligand-induced dimerization of the TLR4 extracellular domains leads to concerted protein conformational changes that in turn lead to self-association or rearrangement of the receptor TIR, thereby creating a new molecular surface for the recruitment of signaling adapter proteins (Núñez Miguel et al., 2007). This model predicts that Mal and TRAM bind to the same region in
the TLR4 dimer interface, thus explaining why cell-permeant blocking peptides compete out both Mal- and TRAM-directed responses simultaneously (Toshchakov and Vogel, 2007). The model does not, however, resolve the question of whether 1) a single activated receptor dimer can stimulate both the Mal- and TRAM-directed pathways simultaneously or 2) adapter engagement is mutually exclusive (something that would require positive cooperativity). Each activated receptor will have two symmetry-related adapter binding sites; in principle, either hypothesis is feasible.

Both Mal and TRAM are regulated by covalent modification. Mal is phosphorylated by Bruton’s tyrosine kinase (Gray et al., 2006). This is required for Mal to signal but subsequently leads to recruitment of suppressor of cytokine signaling 1 (SOCS1) and degradation of Mal (Mansell et al., 2006). Mal also contains a phosphatidylinositol bisphosphate binding domain that localizes it to the plasma membrane (Kagan and Medzhitov, 2006). TRAM is myristoylated (Rowe et al., 2006) in its N terminus, which localizes it to the plasma membrane. It undergoes phosphorylation by protein kinase C-ε, which is required for it to signal. It has also been shown that TLR4 traffics to the early endosome (Kagan et al., 2008); it is here that TRIF is recruited to activate the IRF3 pathway in a manner analogous to the nucleic acid-sensing TLRs (see section V), all of which signal from the early endosomes.

C. Diseases Linked with Toll-Like Receptor 4

The range of ligands (both pathogen-related and endogenous) identified as agonists of TLR4 suggest that this receptor is likely to be associated with a number of diseases. Many published studies suggest that TLR4 is linked to a range of diseases, including infectious disease, atherosclerosis, asthma, cardiac disease, liver disease, renal disease, inflammatory bowel disease, obesity, diabetes (types I and II), rheumatoid arthritis, Alzheimer’s disease, Parkinson’s disease, and multiple sclerosis. Genetic data are emerging to support the association of TLR4 with several of these diseases. Two receptor polymorphisms were originally identified (D299G and T399I) as decreasing responsiveness to inhaled LPS (Arbour et al., 2000). This resulted in a number of studies looking for associations between these polymorphisms and infectious diseases, but much of the data were conflicting (Schröder and Schumann, 2005; Ferwerda et al., 2008). This may be because most of the studies consider either the D299G or the T399I polymorphism but neglect the fact that these polymorphisms also exist in a cosegregated (D299G/T399I) way, which implies that there are four haplotypes: wild type/wild type, D299G/wild type, T399I/wild type, and D299G/T399I (Ferwerda et al., 2008). Recent data suggest that only the D299G haplotype differs in phenotype from wild-type TLR4, LPS-stimulated blood samples from this population of people showing increased, rather than blunted, TNF-α response (Ferwerda et al., 2007).

1. Toll-Like Receptor 4 and Infectious Diseases. Studies in knockout mice have indicated a role for TLR4 in protection against endotoxemia (Hoshino et al., 1999), but an increased susceptibility of TLR4 mutant mice to systemic Gram-negative infections, such as Salmonella typhimurium (O’Brien et al., 1980; Weiss et al., 2004). This is because activation of TLR4 is required for protective immunity against infections but also mediates the effects of systemic endotoxin/infections. Studies of a number of Gram-negative pathogens in mouse infection models have shown a role for TLR4, including Neisseria meningitides, E. coli, Haemophilus influenzae, Klebsiella pneumonae, and Brucella abortus (Schnare et al., 2006). Mouse models have also shown that TLR4 is important for infection with other pathogens, including S. pneumoniae and Mycobacteria tuberculosis (Schnare et al., 2006). TLR4 has also been linked to several viral infections, including respiratory syncytial virus (Kurt-Jones et al., 2000), the murine retroviruses mouse mammary tumor virus and murine leukemia virus (Rassa et al., 2002), as well as the picornavirus Coxackievirus B4 (Triantafilou and Triantafilou, 2004). The role of TLR4 in human infectious disease is emerging. There are now a great number of published studies of polymorphisms in TLR4 and their association with many infectious diseases, including sepsis, Gram-negative infections, other bacterial diseases (including tuberculosis, malaria, and infections with respiratory syncytial virus and Candida spp.) (Ferwerda et al., 2008). The data probably conflict because of the different populations of people studied and the variety of haplotypes involved. The strongest association of TLR4 polymorphisms with an infectious disease is with respiratory syncytial virus infection, where high risk infants heterozygous for D299G and T399I polymorphisms showed an increased susceptibility to infection (Awomoyi et al., 2007). There is also increased risk of severe malaria in Ghanaian children with the TLR4—D299G and TLR4—T399I variants (Mckenhanp et al., 2006) although there is no association between the D299G and tuberculosis in a Gambian population (Newport et al., 2004). An association of the D299G haplotype was found only in the group of patients with septic shock, whereas the D299G/T399I haplotype was found equally in both patients and control subjects, although patients with this genotype had a higher prevalence of Gram-negative infections (Lorenz et al., 2002).

2. Toll-Like Receptor 4 and Noninfectious Diseases. TLR4 and TLR4 receptor polymorphisms have been implicated in a number of noninfectious diseases. This is perhaps unsurprising given the range of endogenous ligands identified for TLR4 and the number of diseases (cancer, atherosclerosis, and autoimmune conditions) that are now believed to have an inflammatory etiology. The D299G SNP is implicated in gastric cancer, athero-
sclerosis, sepsis, and asthma, and a G11481C mutation has been linked to prostate cancer (El-Omar et al., 2008). A number of studies also suggest a possible role for TLR4 in cardiovascular disease (Frantz et al., 2007; Satoh et al., 2008), inflammatory bowel disease (Fukata and Abreu, 2007), Alzheimer’s disease (Balistreri et al., 2008), rheumatoid arthritis (van den Berg et al., 2007), renal disease (Anders et al., 2004), obesity, and diabetes types I and II (Kim, 2006); whether the genetic evidence will support the disease tissue and model observations remains to be proven. In mouse models, for example, inhibition of TLR4 is beneficial in mouse models of rheumatoid arthritis (Eder et al., 2004), and patients with the disease carrying the D299G mutation have altered macrophage responses to LPS (Roelofs et al., 2008), but a clear genetic link between TLR4 and rheumatoid arthritis has yet to be found. It is possible that the genetic data will also be useful in predicting the success of chemotherapeutic regimes (e.g., in cancer chemotherapy). The interaction of HMGB1, released from dying tumor cells, with TLR4 on dendritic cells promoted tumor-specific cytotoxic T-cell responses, and patients with breast cancer who have the D299G polymorphism relapsed earlier after chemotherapy (Apetoh et al., 2008). Whether endogenous ligands or the involvement of infectious disease is the underlying cause of the involvement of TLRs in the susceptibility to these diseases remains to be clarified.

A most interesting recent finding in relation to TLR4 and disease concerns allergy caused by airborne allergens. Derp2, the key allergen from the house dust mite, has been shown to be structurally similar to MD-2 and acts to deliver LPS to TLR4 in airways, thereby provoking inflammation. This might be a common mechanism, because several airborne allergens are lipid-binding proteins and might act analogously. This makes TLR4 a very interesting target for allergy in the airways (Trompette et al., 2009).

3. Myeloid Differentiation Factor 2 and Disease. Studies in the MD-2 knockout mice have been very much more limited compared with the TLR4 knockout mice. Three human polymorphisms have been described: T35A (Hamann et al., 2004), C1625G in the MD-2 promoter (Gu et al., 2007), and G56R (Vasl et al., 2008). The promoter polymorphism may be linked to increased susceptibility to complications such as organ dysfunction and sepsis after major trauma (Gu et al., 2007), whereas the other polymorphisms have yet to show any disease association.

4. Cluster of Differentiation 14 and Disease. A SNP in the 5’ genomic region of CD14 at position 159 (Martinez, 2007) is associated with infectious diseases, asthma, and allergy (Wiertsema et al., 2006). A number of other diseases have also been linked to this polymorphism, from cardiovascular disease to autoimmunity and from infections to malignancies (Martinez, 2007). It would seem that diseases linked to CD14 will overlap those linked to TLR4, and possibly to TLR2, suggesting that therapeutic intervention with either CD14 or TLR4 should benefit patients who have genetic susceptibilities in either of these genes.

D. Pharmacological Manipulation of Myeloid Differentiation Factor 2/Toll-Like Receptor 4

The association of TLR4 with many diseases emphasizes the importance of MD-2/TLR4 as a therapeutic target. A number of different strategies could be considered to pharmacologically alter TLR4, including receptor agonists, receptor antagonists, and signal transduction inhibitors. Some of these are illustrated in Fig. 3. The two discreet signaling pathways (Mal/MD88 and TRAM/TRIF) offer targets for selective modulation of TLR4 activity. The precise clinical goal of modifying TLR4 activity remains an interesting question. In patients with sepsis, for example, it may be better to use a partial agonist rather than an antagonist to decrease TLR4 activity, such that some activation of TLR4 remains to stimulate protective immunity. To regain adjuvant activity, a partial agonist or a drug that selectively stimulates TRAM/TRIF signaling would be safer than a full agonist, which might activate a systemic inflammatory response. Species differences in the response to different agonists at TLR4 suggest that care will be needed in developing safe new drugs.

TLR4 antagonists are currently undergoing clinical trials for treatment of sepsis but may well be useful for the treatment of a range of other conditions. Antagonists

![Diagram of TLR4/MD-2 signaling pathway](image)
of lipid A have been under development since before the discovery of TLRs as treatments for Gram-negative sepsis and endotoxemia. Early work identified a number of lipid A analogs (lipid IVa and Rhodobacter sphaeroides lipid A) (Golenbock et al., 1991). In 1995, a synthetic form of Rhodobacter capsulatus lipid A was generated that antagonized E. coli lipid A and formed the basis for E5531 (Christ et al., 1995). Modification of E5531 generated the stable analog E5564 (eritoran), which is currently undergoing clinical trials for use in treating Gram-negative endotoxemia and sepsis (Mullarkey et al., 2003). E5564 is also able to antagonize the interaction of the protein ligand EDA with TLR4 (Okamura et al., 2003). E5564 is also able to antagonize the interaction of the protein ligand EDA with TLR4 (Okamura et al., 2003), suggesting that this receptor antagonist may be useful for conditions other than sepsis and endotoxemia. Other antagonists at TLR4 include curcumin, auranofin (an antirheumatic gold compound), cinnamaldehyde, and acrolein, all of which prevent homodimerization of TLR4 (Youn et al., 2006a,b, 2008; Lee et al., 2008), although how specific these agents are is uncertain. Small molecules that inhibit MyD88 binding to TLR4 are also emerging (Lee et al., 2007). Cell-penetrating peptides fused with the BB loop sequences of TLR2 and TLR4 also inhibit LPS-induced signaling, probably by interfering with either receptor dimerization or adapter recruitment (Toshchakov et al., 2007). Treatment of patients with sepsis with anti-inflammatory therapies has so far not been beneficial to improving clinical disease (Rittirsch et al., 2008); therefore, it will be very interesting to ascertain the clinical efficacy of inhibiting TLR4/MD-2 activity in sepsis.

TLR4 agonists are currently being developed as immunomodulators and adjuvants. The development of safe and efficacious vaccines remains a major goal in global public health. For these reasons, TLR ligands have become a focus in therapeutic studies for their potential use as adjuvants in vaccine formulations with the systematic development of vaccines that coordinate engage the innate and adaptive immune systems by incorporating a TLR ligand into the vaccine construct. The majority of vaccines comprise two components, the antigen of therapeutic interest and an adjuvant, a component that enhances the immune response to the antigen, a process known as immunogenicity. The nature of adjuvants varies greatly; many elicit adverse side effects. Therefore, the only adjuvant approved and licensed for human use remains limited to aluminum hydroxide. However, the simultaneous delivery of a TLR ligand and an antigen of interest would be more in accordance with natural infection than vaccination with a cocktail of adjuvant and antigen. By physically linking the TLR ligand and antigen, each antigen would be delivered to a vesicle with an activated TLR in a host antigen-presenting cell, potentially achieving optimal antigen processing and presentation (Blander and Medzhitov, 2004; Blander, 2007).

Monophosphoryl lipid A (MPL) was generated by detoxifying Salmonella minnesota lipid A (Qureshi et al., 1982) and was shown to be safe and to have good adjuvant activity. The basis of its adjuvant effect is the stimulation of the TRAM/TRIF signal transduction pathway of TLR4 and deactivation of Mal/MyD88 signaling (Mata-Haro et al., 2007), thereby acting as a partial rather than full agonist at the receptor. MPL has recently been licensed for use as a vaccine adjuvant (Casella and Mitchell, 2008). TLR4 agonists were also generated during the chemical synthetic program to make E5531 and E5564; some of these compounds, such as E6020 (Przetak et al., 2003), have good adjuvant activity (Hawkins et al., 2002). Another class of compounds, the aminoalkyl glucosaminide phosphates (AGP), have been developed as immunomodulators that activate TLR4 (Stöver et al., 2004). Members of the AGP family of lipid A mimetics also have good adjuvant activity and can confer protection against Listeria monocytogenes or influenza virus challenge in the absence of coadministration of the microbe itself or microbial antigen (Cluff et al., 2005).

We have recently found a splice variant of TRAM that we have termed TRAM adapter with gold domain (TAG) (E. Palsson-McDermott, S. L. Doyle, A. F. McGettrick, M. Hardy, H. Husebye, K. Banahan, M. Gong, D. Golenbock, T. Espevik, and L. A. J. O’Neill, manuscript submitted). TAG is localized to late endosomes where it acts to inhibit TRAM and specifically block the MyD88-independent signaling pathway. Interference with TAG would therefore boost this pathway but not the MyD88-dependent pathway. This could be useful in the effort to promote adjuvancy without causing inflammation, because the MyD88-dependent pathway is required for adjuvancy by LPS (Hoebe et al., 2003).

The activation of Mal/MyD88 and TRAM/TRIF signaling pathways offers opportunities to selectively inhibit or activate separate arms of the TLR4 signaling pathway. The success of MPL as an adjuvant in selectively activating the TRIF pathway offers some proof of concept for this pharmacological approach (Mata-Haro et al., 2007). The TLR4 signaling pathways lend themselves to screening with small-molecule inhibitors. The first to be described is the novel cyclohexene derivative TAK-242, a small molecular inhibitor of TLR4 but not TLR2 signaling that probably works by directly inhibiting the intracellular signaling domain of TLR4 (Hi et al., 2006; Kawamoto et al., 2008). Selective agonists and antagonists of TLR4 signaling are likely to be potent therapeutic compounds. The compounds currently available have largely been restricted to being used as antagonists in patients with sepsis and as adjuvant, but as our understanding of the role of TLR4 in disease is clarified, it is likely that these compounds will be useful in a range of diseases. The availability of structural data for TLR4 and MD-2 is likely to speed up the process of compound design and production.
III. Toll-Like Receptor 2: Agonism and Antagonism

TLR2 was originally identified as the LPS receptor (Kirschning et al., 1998; Yang et al., 1998), but the TLR2 activation by LPS was subsequently attributed to the bacterial lipoprotein contamination of the LPS preparations (Lee et al., 2002; Hellman et al., 2003). TLR2 recognizes a wide range of ligands, many of which are from Gram-positive bacteria (Takeuchi et al., 1999), and it signals not as a homodimer but as a heterodimer with either TLR1, TLR6 (Ozinsky et al., 2000; Takeuchi et al., 2001), or TLR10 (Hasan et al., 2005). Mice without TLR2 are hyporesponsive to Gram-positive bacterial cell wall components (Takeuchi et al., 1999). A number of SNPs in TLR2 have been reported in the extracellular domain [R753Q (Lorenz et al., 2000), Y715K, and Y715stop (Merx et al., 2007)] and the cytoplasmic domain [P631H (Smirnova et al., 2003)]; although the extracellular domain mutations result in decreased activity of TLR2 to Gram-positive bacterial ligands (Lorenz et al., 2000; Schröder et al., 2003), the genetic evidence linking these SNPs with a susceptibility to Gram-positive infections is unclear (Schröder and Schumann, 2005). Twelve SNPs in TLR1 [of these, S248N, H305L, P315L (Johnson et al., 2007), and 1602S (Johnson et al., 2007) have defective signaling] and 14 SNPs in TLR6 (Johnson et al., 2007) have also been identified. It is unclear whether TLR2 heterodimerization leads to the activation of differential signaling, and although this is an attractive hypothesis, there is currently no evidence to support it (Farhat et al., 2008). TLR2 also cooperates with other receptors for pathogen recognition; for example, cooperation between TLR2 and Dectin 1 allows recognition of the yeast particles such as zymosan (Brown et al., 2003; Gantner et al., 2003). In a manner analogous to TLR4, other proteins have been implicated as coreceptors for TLR2, such as CD14 (Jiang et al., 2005) and CD36 (Hoebe et al., 2005).

A. Ligand Recognition at Toll-Like Receptor 2

A wide range of structurally diverse ligands, including those derived from microbial, fungal, and endogenous sources, is believed to be recognized by TLR2 (Zähringer et al., 2008). Many of these ligands are glycolipids, lipopeptides, or glycosylphosphatidylinositol-based structures [e.g., lipoteichoic acid (LTA) from Gram-positive bacteria, lipoarabinomannan from mycobacteria, and glycosylphosphatidylinositol-based lipids from Trypanosoma cruzi] that, like LPS, contain a significant hydrophobic component (Zähringer et al., 2008). Well characterized ligands of TLR2 include Mycoplasma fermentans macrophage activating lipopeptide (MALP-2), which interacts with TLR2/TLR6 (Takeuchi et al., 2001), triacylated lipopeptides (such as Pam3CSK4 at TLR2/TLR1), and diacylated lipids/lipopeptides (LTA, Pam2CSK4) (Ozinsky et al., 2000; Takeuchi et al., 2001). Like the recognition of LPS by TLR4, the number of acyl chains linked to a lipoprotein plays an important role in ligand recognition by TLR2. Other properties of lipoproteins, such as the ester bonds present in the acyl chains and the nature of the amino acids, are also discriminated by TLR2 heterodimers (Buwitt-Beckmann et al., 2005a,b), and some lipopeptides may be recognized by TLR2 independently of TLR1 or TLR6 (Buwitt-Beckmann et al., 2006).

The molecular basis for lipoprotein recognition by TLR2 was largely explained by the solving of the crystal structure of the TLR1/TLR2 heterodimer bound to Pam3CSK4 (Jin et al., 2007). In this structure, the extracellular domains of TLR1 and TLR2 form an “M”-shaped heterodimer, with the two N termini extending outward in opposite directions. The lipid chains of Pam3CSK4 bridge the two TLRS, therefore playing a crucial role in the formation of the heterodimer. Two of the three lipid chains of Pam3CSK4 interact with a hydrophobic pocket in TLR2, and the amide-bound lipid chain lies in a hydrophobic channel within TLR1. The ligand-bound complex of TLR1 and TLR2 is stabilized by protein-protein interactions at the interface near the ligand-binding pocket. The TLR1 Pro315Leu polymorphism is located at the TLR1-TLR2 interface, which probably explains why it reduces TLR1/TLR2 signaling (Jin et al., 2007). This structure also explains mutagenesis data identifying the leucine-rich repeat region 9 to 12 of TLR1/TLR6 as being important in recognizing lipopeptides (Omueti et al., 2005; Andersen-Nissen et al., 2007). The nature of ligand binding at TLR2/TLR6 is unknown, but presumably it will be similar to that at TLR1/TLR2. How coreceptors such as CD14 or CD36 fit in with the structural data remains unclear. CD36 contributes to diacylglyceride recognition only at TLR2/TLR6 (Hoebe et al., 2005); it is unknown how CD14 interacts structurally with the MD-2/TLR4 complex, but CD14 is critical for TLR4 signaling. It may be some time before it is clear how coreceptors such as CD14 and CD36 interact with TLR2 signaling complexes.

The data on lipoprotein recognition at TLR2 are clear but do not explain why this receptor recognizes such a wide array of different ligands. Peptidoglycan and LTA both bind to TLR2, but precisely which structures in these ligands interact with the receptor is unclear. LTA, like LPS, is a glycolipid with repeating carbohydrate units from Gram-positive bacteria, and it has been suggested that the d-alanylation in the Gro-P repeating units of this lipid are recognized by TLR2 (Morath et al., 2002), although this is controversial (Zähringer et al., 2008). TLR2 is one of several receptors believed to be important for peptidoglycan recognition, but the structural motif in peptidoglycan recognized by TLR2 is unknown. It is possible that the peptidoglycan recognition attributed to TLR2 is due to contamination of the peptidoglycan preparation with lipoproteins (Zähringer et al., 2008). A range of LPS structures has also been
suggested to be TLR2 ligands, such as lipid A from Porphyromonas gingivalis (Hirschfeld et al., 2001), Legionella pneumophila (Girard et al., 2003). This remains a controversial topic. Contaminating lipoproteins in the LPS from P. gingivalis could explain why the LPS from this bacterium can activate TLR2, but results from studies of this subject remain confusing (Hashimoto et al., 2004). Given the structural data, however, it is difficult to understand how LPS structures with more than three acyl chains, such as the LPS from P. gingivalis, could be accommodated within the TLR2 binding site. The molecular mechanisms underlying how ligands such as whole bacteria [e.g., Francisella tularensis (Cole et al., 2007) and fungal ligands [e.g., zymosan (Ganttner et al., 2003)] are recognized by TLR2 or TLR2/dectin 1, respectively, remain unknown.

B. Efficacy at Toll-Like Receptor 2: Recruitment of Adapter Proteins

There is clear evidence to support the TIR domain of TLR2 heterodimerizing with the TIR of TLR1 or TLR6 to induce signaling (Ozinsky et al., 2000). TLR2 activates only MyD88-dependent signaling and requires Mal to recruit MyD88 to the TIR domain (Fitzgerald et al., 2001; Horng et al., 2001, 2002; Yamamoto et al., 2002). This means that TLR2 signaling induces expression of only a subset of genes activated by TLR4 (Hirschfeld et al., 2001). There are limited data on how the TIR domains of TLR2 and TLR1 or TLR6 interact. One study used molecular modeling and mutagenesis analysis of the TLR2/TLR1 heterodimer and showed that 4 residues are important: Arg748, Phe749, Leu752, and Arg753. The model suggested that, of these residues, Arg748 and Phe749 in TLR2 (in the DD loop) were in close contact with Gly676 from TLR1 (in the BB loop); and mutation of Gly676 or Gly676 in TLR1 or Gly676 in TLR2 reduced Pam3CSK4-induced signaling (Gautam et al., 2006); however, further structural work is required to verify these observations. A similar analysis has not been performed for TLR2/TLR6. The regulation of signaling via Mal and MyD88 is likely to be similar to that induced by TLR4.

C. Diseases Linked with Toll-Like Receptor 2

The range of ligands recognized by TLR2 would suggest that this receptor is likely to play an important role in many diseases and therefore be a useful therapeutic target.

1. Toll-Like Receptor 2 and Infectious Disease. An early phenotype of the TLR2 knockout mice was an increased susceptibility to high-dose but not low-dose infection with Staphylococcus aureus (Takeuchi et al., 2000). Subsequent studies suggest that TLR2 plays a role in infection pathogenesis with other Gram-positive pathogens (S. pneumoniae meningitis, group B streptococcus, Bacillus subtilis, L. monocytogenes), and other bacterial species (Chlamydia trachomatis, spirochetes, Mycobacterium tuberculosis, and Yersinia enterocolitica) (Marra and Brigham, 2001). Viral (herpesvirus (Compton et al., 2003; Kurt-Jones et al., 2004) and paramyxovirus (Bieback et al., 2002)) and fungal diseases have also been linked to TLR2. The first SNP [in the C-terminal region of human TLR2 (R753E)] was identified in 2000 (Lorenz et al., 2000). This polymorphism had decreased activation by TLR2 ligands when transfected into human embryonic kidney 293 cells (Lorenz et al., 2000). Similar to the frequency of the TLR4 polymorphisms D299G and T399I (among white persons), the occurrence of the R753E SNP as homozygote seems to be rare [e.g., 9.4% heterozygous for R753E and no homozygotes in a white population (n = 319)] (Schroder et al., 2003). The R753E SNP is now no longer thought to be associated with the severe diseases caused by S. aureus (Moore et al., 2004), but may be associated with Candida spp. sepsis (Misch et al., 2008). The R753E SNP does seem to be linked to tuberculosis (Ogus et al., 2004) and also to acute rheumatic fever in children (Berdelli et al., 2005) and end stage Lyme disease (Schroder et al., 2005). An R677W SNP, a mutation that also decreases TLR2 activation by TLR2 ligands in vitro, has been shown to be associated with lepromatous leprosy in Asian people, but this SNP is now believed to be a polymerase chain reaction artifact from a TLR2 pseudogene (Malhotra et al., 2005). The TLR2 P631H SNP is less frequent in people with meningitis (Srinovana et al., 2003). The TLR1 I602S SNP not only decreases signaling to TLR2 ligands but also may prevent trafficking of this receptor to the cell surface and is associated with susceptibility to meningeal tuberculosis and leprosy (Hawn et al., 2006; Johnson et al., 2007). In Nepalese patients with leprosy, the 1805G allele of TLR1 is associated with protection against leprosy (Misch et al., 2008). Hypermorphic genetic variation in TLR1, particularly the G allele of TLR1–7202A/G (rs5743551), is associated with increased susceptibility to Gram-positive infection in sepsis (Wurfel et al., 2008).
2008). The genetic evidence linking diabetes types I and II to TLR2 is controversial (Park et al., 2004; Santin et al., 2006), but experimental evidence suggests that TLR2 may be a useful therapeutic target in some forms of this disease (Caricilli et al., 2008). TLR2 may also be important in the pathogenesis of renal disease, particularly in infection and toxic injury (Anders et al., 2004). There is also some evidence to link a TLR2 guanine-thymine microsatellite repeat polymorphism in the second intron to susceptibility to sporadic colorectal cancer (Boraska Jelavic et al., 2006).

TLR2 has also recently been implicated in SLE and also in ischemia/reperfusion injury in kidney (Leemans et al., 2005; Urbanaviciute et al., 2008). Prostate cancer has also been linked to TLR2 through its association with TLR10 (Kormann et al., 2008; Stevens et al., 2008).

**D. Pharmacological Manipulation of Toll-Like Receptor 2**

Currently, the major use for compounds that activate TLR2 are as adjuvants. The synthetic compounds, such as Pam3CSK4 and MALP-2, could be developed for adjuvant usage. TLR2 should be a useful therapeutic target for the development of antagonists given the range of diseases with which this receptor is associated. A series of novel synthetic phospholipids that are antagonists at TLR2 have been made, but there are few data on the use of these compounds (Spyvee et al., 2005). Inhibition of TLR2-induced signaling through Mal/MyD88 would also result in partial inhibition of TLR4 signal transduction. This might be a useful approach, particularly targeting Mal, given that it binds exclusively to TLR2 and TLR4. A number of diseases, such as sepsis, diabetes, rheumatoid arthritis, and cardiovascular diseases, seem to be linked to both TLR2 and TLR4; therefore, Mal seems to be an attractive therapeutic target for these diseases.

Another approach to blocking TLR2 is with a neutralizing antibody. One such antibody, T2.5, has been shown to prevent sepsis induced by TLR2 ligands (Meng et al., 2004); furthermore, when T2.5 is used in combination with an anti-TLR4/MD-2 antibody, it protects mice against sepsis induced by Salmonella enterica or E. coli when given with antibiotics (Spiller et al., 2008). This latter finding suggests that a combination approach involving anti-TLR4 and anti-TLR2 might be a very useful adjunct to antibiotics in the prevention of sepsis.

**IV. Toll-Like Receptor 5: Agonism and Antagonism**

TLR5 is the receptor for bacterial flagellin monomers and is the only TLR that recognizes a protein ligand (Hayashi et al., 2001). The region of flagellin that TLR5 recognizes is highly conserved among microbial species and therefore allows TLR5 to detect a wide variety of microbes. TLR5 signals by recruiting the TIR adapter MyD88, leading to the activation of the IKK complex and subsequent activation of the proinflammatory transcription factor NFκB, which in turn results in the increased expression of pro-inflammatory genes. In addition, TLR5 ligation activates a number of antiapoptotic genes, allowing cells to stay alive in response to challenges that would otherwise result in cell death. In this way, TLR5 activation is cytoprotective (Zeng et al., 2006). This characteristic of TLR5 has recently been harnessed to protect cells against ionizing radiation. Ionizing radiation, the primary therapy for cancer patients, is a double-edged sword. Although it destroys tumor cells, it also causes healthy cells to undergo apoptosis. Tumor cells can persist because they can block apoptosis by activating NFκB, and much of the research in the cancer field involves developing methods to inhibit NFκB in tumor cells. Burdelya et al. (2008) have instead attempted to activate NFκB in normal cells to give these radiosensitive tissue cells a higher chance of surviving radiation therapy by suppressing apoptosis. The toxicity of high-dose ionizing radiation is associated with the induction of acute radiation symptoms involving the hematopoietic system and the gastrointestinal (GI) tract. To activate NFκB in these radiosensitive cells without inducing acute inflammatory responses, Burdelya et al. (2008) focused on TLR5 because of its activation in the gut in response to the protective role played by benign commensal microorganisms in the GI tract. An engineered flagellin derivative named CBLB502 was found to have potent NFκB activation and reduced immunogenic characteristics. A single injection of CBLB502 before lethal total body irradiation protected mice and rhesus monkeys from both GI and hematopoietic acute radiation symptoms and resulted in improved survival and yet, importantly, did not decrease tumor radiosensitivity. These results imply that TLR5 agonists may be valuable as adjuvants for cancer radiotherapy (Burdelya et al., 2008).

The activation of TLR5 has also been recently reported to be an efficient adjuvant for an influenza A vaccine. Development of influenza vaccines is challenging because of the genetic instability of the commonly used antigens hemagglutinin and neuraminidase; therefore, these vaccines require annual reformulation. A promising genetically stable influenza antigen is the ectodomain of the matrix2 protein (M2e). However, although the sequence of M2e is stable across all influenza A isolates, dating as far back as a pandemic strain in 1918, studies have shown M2e to be poorly immunogenic. As described previously, immunogenicity of an antigen can be improved upon by the delivery of the antigen in combination with adjuvant. However, in the past, several strategies have been tested in an attempt to produce a universal influenza vaccine incorporating the conserved antigen M2e, but its poor immunogenicity could not be overcome with any efficacy. However, M2e was recently fused with the TLR5 ligand S. typhimurium flagellin (STF2). The resulting fusion protein can activate cells in a TLR5-dependent manner and
elicits potent antibody responses in mice. This study demonstrates that a recombinant protein containing a consensus M2e sequence linked to the TLR5 ligand provides an effective approach to developing vaccines against wide-spread epidemic and pandemic influenza (Huleatt et al., 2008). These findings indicate that activating the TLR5 signaling pathway may have broad therapeutic applications, not only in its role as a linker adjuvant candidate for vaccines, but also as a damper of excessive apoptosis in acute radiation syndromes, a characteristic that may be extended for use in degenerative diseases and ischemia reperfusion injury as well.

However, the over-activation of TLR5 may have a negative effect on certain diseases of the gut. Crohn’s disease and ulcerative colitis, two related chronic inflammatory diseases, are known collectively as inflammatory bowel disease (IBD). IBD is thought to be the product of a combination of genetic and environmental factors that result in the abnormal regulation of the immune responses. Experimental studies suggest that IBD is a T-cell driven process that results from defects in the T-cell-mediated regulatory processes that would normally prevent and or terminate inflammatory responses (Himmel et al., 2008). There is also strong evidence to suggest that abnormal responses to commensal bacteria are also central to the development of IBD (Strober et al., 2008). The GI tract is a unique organ, in that although it maintains the ability to mount an immune response to pathogens, it needs to remain tolerant to dietary antigens and commensal bacteria. Many studies have indicated that CD4+ T-regulatory cells expressing forkhead box P3 (FOXP3) or IL-10 have a fundamental role in maintaining gut immune homeostasis (Himmel et al., 2008); however, TLRs are also thought to be necessary for maintaining tolerance (Crellin et al., 2005). In the case of IBD, TLRs can also amplify inappropriate immune responses that ultimately cause chronic inflammation. Accumulating data suggest that TLR stimulation on T cells, presumably by commensal bacteria, has a significant role in the development of IBD (Himmel et al., 2008). Additional evidence for the role of TLRs in IBD comes from studies of flagellin and TLR5. A common polymorphism in TLR5 that produces a dominant-negative receptor is protective against Crohn’s disease (but not ulcerative colitis) (Gewirtz et al., 2006). This is in contrast to mice, where TLR5-deficient mice develop spontaneous colitis, suggesting that TLR5-flagellin ligation would normally have a protective role (Vijay-Kumar et al., 2007), this difference may be due to the differential expression of TLR5 in mice and humans. Further studies have indicated that a difference in the concentration of flagellin can have opposite effects on T-cell function, suggesting a model for how flagellin could influence the balance between regulatory and effector T cells. At low concentrations, flagellin can stimulate TLR5 on CD4+ T cells and enhance the expression of FOXP3, allowing for an increased suppressive capacity of regulatory T cells, whereas high concentrations stimulate T-effector function (Crellin et al., 2005). In the case of IBD, the latter would probably prevail with increased effector cell levels, causing the eventual loss of all regulatory T cell function. Because of the ability of the human TLR5 mutation to protect against Crohn’s disease, pharmacological targeting of this pathway antagonistically may turn out to be therapeutically beneficial.

V. Toll-Like Receptors That Recognize Nucleic-Acid Ligands

TLRs 3, 7, 8, and 9 are all nucleic acid-recognizing TLRs expressed on endosomal membranes. Nucleic acid recognition has so far been resolved for double-stranded RNA binding to TLR3. Each extracellular domain of TLR3 binds to dsRNA at two sites located at opposite ends of the TLR3 horseshoe. There is also an intramolecular contact between the two TLR3 extracellular C-terminal domains that coordinates and stabilizes the TLR3 dimer. This structural arrangement can then mediate downstream signaling by dimerizing the cytoplasmic TIR domains. If there is a common mechanism for nucleic acid recognition at TLRs, then recognition of nucleic acids differs from that of the bacterial lipids quite considerably (Liu et al., 2008).

Cytokine profiles produced by activated TLRs are dependent on the type of ligand they recognize, which allows for an immune response fine-tuned for each microbe. In general, however, nucleic acid-based agonists of TLRs induce Th1-type immune responses (Agrawal and Kandimalla, 2007).

A. Toll-Like Receptor 3 Activation in Vaccine Adjuvancy and Antitumor Immunity

TLR3 has been shown to be the receptor for viral dsRNA and the dsRNA mimic poly(I:C) (Alexopoulou et al., 2001). Most viruses synthesize dsRNA at some point during their replicative cycle; therefore, TLR3 is an important detector of viral infection and initiator of the antiviral immune response. Once activated, TLR3 signals through its TIR adapter TRIF to both the IKK complex IKKα/IKKβ/IKKγ and the noncanonical IKK complex of TBIK/IKKe, culminating in the activation of proinflammatory and antiviral transcription factors. This signal cascade leads to changes in gene expression in a Th1-type pattern, resulting in the activation and maturation of antigen-presenting cells such as dendritic cells (DCs) and monocytes, allowing for the regulated processing and presentation of antigens, the up-regulation of major histocompatibility complex, and costimulatory molecules and secretion of proinflammatory chemokines and cytokines. These events then mediate the activation of antigen-specific T- and B-cell responses. In these ways, TLR signaling mediates an effective immune response by contributing to the priming and the type of the adaptive immune response. Because DCs are
potent inducers of T-cell-mediated immunity and are activated through TLR ligation, they are attractive targets to improve vaccine efficacy.

Subsequently, a potential therapeutic use for stimulating TLR3 with an agonist is that of vaccine adjuvancy. Poly(I:C) shows potential as an adjuvant for DC-targeted vaccines. One group interested in improving the efficacy of T-cell-mediated immunity induced by HIV vaccines has demonstrated that when vaccine antigens were administered along with poly(I:C) protective specific CD4+, T-cell immunity was induced and was long-lasting in a lung infection model (Trumpfheller et al., 2008). It has also recently been reported that mDCs exposed to TLR3/TLR4 ligands up-regulated their expression of 1α-hydroxylase, an enzyme that converts the precursor of vitamin D₃ precursor into calcitriol, the active form. Calcitriol-dependent mechanisms allow for the ability of DCs to migrate from skin sites of vaccination to mucosal lymphoid organs. Therefore, vaccines containing TLR3/TLR4 ligands and the specific antigen administered subcutaneously can function as effective mucosal adjuvants because they stimulate the metabolism of vitamin D₃ (Enioutina et al., 2008).

Activation of TLR3 is also potentially promising as an anticancer therapy. Th1 immune activation is optimized for fighting intracellular infection such as viruses and involves the activation of natural killer (NK) cells and T cells that can lyse infected cells. This Th1-pattern of cytokine and chemokine secretion is highly desirable for cancer therapy. TLRs can be expressed on cancer cells and are therefore implicated as possible options in cancer-based therapy. However, the expression patterns of TLRs in human cancer tissues are largely unknown; studies to date stimulating cancer tissues with TLR agonists have shown that the effects of stimulation may be either pro- or antiapoptotic in different cases. An example of this is the ligation of TLR9; although it inhibits the progression of growth in prostate cancer cells, it causes increased growth in breast cancer cells (Huang et al., 2005). A TLR3 agonist, however, may prove useful as an anticancer agent in a number of cases, because functional TLR3 has been shown to be expressed in breast cancer cells and to a very high extent in both primary and metastatic clear-cell renal cell carcinoma, one of the most drug-refractory cancers known. Poly(I:C) induced TLR3-dependent IFNα production and exerted a growth inhibitory effect against clear-cell renal cell carcinoma cells and breast cancer cells. These studies are examples of reports that cancer cells themselves express functional TLR3 in vivo and that poly(I:C) is acting directly through them and not solely through the stimulation of DCs leading to anticancer effects (Salaun et al., 2006). Furthermore, retrospective analysis of an old clinical trial of a TLR3 ligand, poly-AU, in breast cancer patients has shown that there was an improved survival rate in the subset of patients whose tumor cells themselves expressed TLR3 (Andre et al., 2004). It is noteworthy that the adverse effects of administering TLR3 agonists to cancer patients might be limited; although there is a high level of TLR3 expression in these cancer cells, there is low TLR3 expression elsewhere in the body.

Although TLR3 activation may be desirable in increasing vaccine efficacy and as an anticancer therapy, it can be an unwanted contraindication of a different therapeutic mechanism. RNA interference (RNAi) or RNA silencing uses dsRNA to exploit a natural antiviral intracellular pathway to induce the knockdown or “silencing” of a specific gene. The silencing mechanism is triggered when dsRNA interacts with the endoribo-nuclease Dicer, which cleaves the aberrant dsRNA into 21-base pair fragments; these are known as small interfering RNA (siRNA), which are incorporated into an RNA-induced silencing complex that unwinds the siRNA and anneals it to the complementary RNA target, subsequently cleaving and degrading it. The use of nucleic acid-induced gene silencing, such as RNAi, as a therapy has been a focus since the discovery of the antiviral machinery for silencing specific target genes in the late 1990s (Fire et al., 1998) and has led to the hope that specific diseases may benefit therapeutically from knockdown of a single gene, especially when initial studies suggested that in contrast to long dsRNA, siRNA did not induce a nonspecific antiviral response (Karpala et al., 2005). In fact, Reich et al. (2003) and Shen et al. (2006) reported that siRNAs targeting VEGFα or the VEGF receptor 1 effectively inhibited ocular choroidal neovascularization ( CNV) in mouse models. CNV occurs at a late stage in age-related macular degeneration (which afflicts 30 to 50 million people globally), in which the retina is invaded by choroidal vessels, causing blindness. Because of these studies, initial clinical trials were set up using intracocular injection in patients with CNV. More recently, however, various groups have demonstrated that the antiviral response is activated upon introduction of siRNA implicating TLR3 activation by siRNA (Karioko et al., 2004). In fact, Kleinman et al. (2008) have since claimed that suppression of neovascularization is a generic property of siRNAs independent of sequence, target, and internalization. They showed that numerous synthetic nontargeted 21-nucleotide duplex siRNAs, siRNAs targeting nonmammalian genes, or nuclease digestion abolished the angioinhibitory effect of siRNA-induced CNV suppression, a chemically modified siRNA unable to interact with the RNA-induced silencing complex could no longer effect CNV inhibition. Kleinman et al. (2008) clearly show that siRNA suppression of CNV, whether using targeted siRNA against VEGFα/VEGF receptor 1 or nontargeting sequences, is via TLR3 activation, because nontargeting siRNA could not suppress CNV in Tlr3(−/−) mice, whereas poly(I:C) and dsRNA
were shown to suppress CNV in wild-type mice but not in Tlr3 (−/−) mice. The angioinhibitory effect was similarly shown to require the TIR adapter TRIF and the induction of IL-12 and IFN-γ, both known for their antiangiogenic abilities. It is possible that a difference in the method of siRNA introduction could explain the different results observed between the study by Reich et al. (2003) and that by Kleinman et al. (2008). The siRNAs in the former were injected subretinally, whereas in the latter, the siRNA was introduced by intravitreous injection. It is more probable, however, that the difference is due to the differing lengths of siRNA used by Reich et al. (2003). Kleinman et al. (2008) show that the siRNA needs to be a minimum of 21 nt to engage and activate TLR3; however, although the VEGF-targeting siRNA in Reich’s study was 21 nt long, EGFP-targeting siRNA was used as the control in this case, and although the antisense strand of this siRNA was 21 nt long, the sense strand was only 18 nt. This would effectively halve the dose of TLR3-activating siRNA available in the control experiments. The discrepancies between the study by Kleinman et al. (2008) and that of Shen et al. (2006) are more difficult to reconcile because the studies used the same method of introduction and the siRNAs were 21 nt long. The differences again are likely to come down to choice of control siRNA. Shen et al. (2006) show data for one control siRNA, whereas Kleinman et al. (2008) describe 12 control siRNAs. However, why the control siRNA used in by Shen et al. (2006) did not activate TLR3 and inhibit angiogenesis whereas those used by Kleinman et al. (2008) study did remains unclear. The study by Kleinman et al. (2008) has high-reaching implications for the development of siRNAs as a method for treatment of disease in general, in that they may induce unanticipated vascular or undesirable antiviral effects. However, it was necessary for the siRNA molecules to be at least 21 nt in length to induce a TLR3 response; therefore, it might be possible to enhance the therapeutic specificity of siRNAs and abrogate TLR3 activation by reducing the siRNAs to under 21 nt (Kleinman et al., 2008). Nonetheless, therapeutic use of RNAi needs careful consideration because there is potential for wider impact on the system as a result of the associated stimulation of the antiviral signaling pathways.

B. A Potential Case for Toll-Like Receptor 3 Antagonism

TLR3 antagonism may be beneficial in treating West Nile virus (WNV) infection. Infection of macrophages or DCs by WNV in peripheral lymphoid tissue induces TLR3-dependent secretion of TNFα and results in a transient increase in the permeability of the blood-brain barrier (BBB), facilitating the penetration of WNV across the BBB and into the CNS. Although the exact mechanism of entry into the CNS remains unclear, TNFα may alter endothelial cell tight junctions allowing either WNV itself across the BBB or leukocytes carrying the virus to pass between the endothelial cells of the BBB; it is clear, however, that that TLR3 activation is vital to the passage of the virus into the CNS. TLR3-deficient mice have an increased survival rate after WNV infection, lower viral titers in the brain, and decreased BBB leakiness as a result of reduced levels of TNFα (Wang et al., 2004). Therefore, inhibition of TLR3 signaling and the subsequent reduction in TNFα could be a potential means of treating persons infected with WNV.

C. Toll-Like Receptors 7 and 8: Small-Molecule Targets

As mentioned previously, DCs are essential for mediating an effective immune response. There are two types of DCs: myeloid DCs (mDCs) and plasmacytoid DCs (pDCs). pDCs are particularly important in the antiviral response because of their ability to produce large amounts of type I IFNs upon viral infection. This function of human pDCs is due to their selective expression of TLR7 and TLR9. TLR8, however, is preferentially expressed by mDCs and monocytes. Like TLR3, TLRs 7 and 8 are antiviral TLRs. In this case TLRs 7 and 8 recognize single-stranded RNA sequences containing GU-rich or poly-U sequences as their natural ligands, but they are also activated by synthetic small-molecular-weight compounds of the imidazoquinoline family, such as resiquimod and imiquimod (Lee et al., 2003; Diebold et al., 2004; Heil et al., 2004). Upon ligation, TLRs 7 and 8 recruit the TIR adapter MyD88 and initiate a signaling cascade, resulting in the activation of both pro-inflammatory transcription factors such as NFκB and antiviral factors such as the IRF family of transcription factors. They are localized intracellularly to endosomal membranes and act as potent activators of innate immune responses upon viral infection. So, it is not surprising that the therapeutic potential in targeting TLR7 and 8 has focused on developing TLR7/TLR8 agonists as “antiviral agents.” Imidazoquinolines were originally developed as such antiviral agents, and many such small-molecule compounds have been tested for their ability to induce TLR7/TLR8-mediated cytokine induction. Imiquimod is the first approved topicaly active TLR7 agonist. It is prescribed for treatment of external virus-induced skin lesions, such as the genital and perianal warts resulting from papillomavirus infections (Gupta et al., 2004). Although the exact mechanism of action is unknown and may involve other receptors, cellular responses to imiquimod include the induction of cytokines such as IFNα, IL-12, and TNFα and chemokines such as IL-8, macrophage inflammatory proteins 1α and 1β, and macrophage chemotactic protein 1. Production of such mediators allows for the activation of and attraction of effector cells such as DCs and cytotoxic T cells to the lesion site. NFκB-dependent IFNγ induction and the induction of 2',5'-oligoadenylate synthetase has also been demonstrated resulting in the stimulation of NK

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cells, which are integral to antiviral and antitumor activity (Miller, 2002; Navi and Huntley, 2004).

Therapeutic interest in TLR7/TLR8 for cancer treatment came about because of the antitumoral activity of TLR7/TLR8 agonists (Sidky et al., 1992). As such, imiquimod is now also used as a treatment for cancer and has shown itself to be efficacious against primary skin tumors and cutaneous metastases (Schön and Schön, 2008). In fact, imiquimod has been approved for therapeutic use in a number of oncological and virus-associated diseases, including external genital warts, precancerous actinic keratoses, basal cell carcinomas (the most common of skin cancers), and on lesions formed as a result of metastatic melanoma. In addition, it has been shown to be an effective treatment for herpes simplex virus in some cases, offering an alternative therapy for persons who are resistant to conventional treatment (Gupta et al., 2004; Miller et al., 2008). Hepatitis C virus (HCV) has also been under investigation as a potential target disease that would benefit therapeutically from TLR7 agonists. Chronic HCV infection affects 3% of the world’s population, and the current therapy is based on a combination of pegylated IFNα and the nucleoside analog ribavirin (Schultheiss and Thimme, 2007). However, less than 50% of the population responds to this treatment, and novel strategies for treating HCV are therefore needed. A number of studies suggest that activation of TLR7 would be beneficial in persons infected with HCV. Indeed, one study has shown that TLR7 is expressed in normal and HCV-infected hepatocytes, and activation of TLR7 alone reduces HCV mRNA and protein levels (Lee et al., 2006). This was associated with stimulation of IRF7 and could not be neutralized by the addition of an IFNα receptor antibody, suggesting that TLR7 ligation can inhibit HCV replication by direct activation of antiviral mechanisms within the hepatocytes and not just via IFNα production. Further evidence of a role for TLR7 comes from a study demonstrating that a variant TLR7, due to a SNP c.1–120T>G, was associated with less inflammation and liver fibrosis in male patients. The increased IL-6 secretion observed in peripheral blood mononuclear cells from these patients is associated with a decrease in fibrosis progression. Because TLR7 is located on the X chromosome, it is not surprising that this effect was only observed in male patients expressing the variant (Schott et al., 2007). These combined studies clearly support a role for TLR7 in natural HCV infection. However, a study was carried out in which patients were administered resiquimod orally, and although low doses were well tolerated, they had little antiviral effect; higher doses seemed to decrease HCV levels after the first dose, but the higher dosing resulted in severe side effects (Pockros et al., 2007). To date, patients have responded best to the injectable TLR7 agonist isatoribine in a proof-of-concept study that resulted in reduced viral load in chronically infected patients. An oral prodrug of isatoribine, ANA975, was developed for the treatment of chronic HCV infection and initial results were promising (Hornsman et al., 2005); however, further trials were suspended when toxicity developed in long-term animal studies (Fletcher et al., 2006). Further studies are therefore necessary to determine whether there will be any advantage of TLR therapy over the current option.

An aqueous imidazoquinoline that could be administered systemically would be potentially important for the treatment of severe diseases such as chronic lymphocyte leukemia and solid-tumor cancers. Recent studies have focused on improving the stability of the single-stranded RNA-based TLR7/TLR8 agonists and enhancing their delivery for systemic use (Agrawal and Kandimala, 2007). The resulting stabilized immune modulatory RNA compounds have since been shown to induce T cell, monocyte, and NK cell activation and migration and to induce elevated levels of Th1-type cytokine in the plasma of non-human primates (Lan et al., 2007). Another group has synthesized a TLR7 agonist that can be coupled to many different chemical entities, potentially allowing for directed systemic use of this TLR7 agonist (Wu et al., 2007). Yet another group has synthesized an aqueous imidazoquinoline, known as 852A, that is potentially promising for treating cancers of the blood (Dudek et al., 2007).

D. Toll-Like Receptor 9

At present, TLR9 is the only TLR for which a systematically administered specific agonist has shown substantial evidence of antitumor activity in human clinical trials. TLR9 has evolved to recognize unmethylated CpG dinucleotides (CpG ODN) that are prevalent in viral and bacterial DNA but not in vertebrate genomes. TLR9 is expressed on B cells and pDCs in humans and is pivotal to the production of type I IFN, which in turn is essential to control viral replication and eradicate infected cells. CpG ODNs are spontaneously taken up by most immune cells; upon uptake, TLR9 translocates to the same compartment, allowing for ligation and initiation of TLR9-dependent signal transduction. TLR9 stimulation activates innate immunity in a predominantly Th1 pattern. In this way, TLR9 activation is very similar to TLR3/TLR7/TLR8 activation and results in increased expression levels of costimulatory molecules such as TNF-related apoptosis-inducing ligand (TRAIL), which can induce tumor cell death, and CC chemokine receptor 7, which when activated causes cell trafficking to the T-cell zone of the lymph nodes. Together, these innate immune effects of TLR9 activation can promote tumor regression directly through the action of TNF-related apoptosis-inducing ligand or indirectly through the activation of NK cell-mediated tumor killing (Krieg, 2007).

The pattern with which CpG ODNs activate the immune system suggests the use of such TLR9 agonists as effective vaccine adjuvants. Indeed CpG ODNs have been shown to be beneficial in animal models as a vaccine adjuvant not only for infectious diseases (Jurk and
Vollmer, 2007), but also as a tumor vaccine adjuvant. CpG ODNs seem to be the most promising of all adjuvants currently in preclinical development; they induce a Th1 environment and can synergize with other adjuvants, such as liposomes, which allow for enhanced uptake (Jurk and Vollmer, 2007). In fact, not only are CpG ODNs excellent adjuvants for normal therapeutic vaccination, but they also have the ability to help vaccine-hyporesponsive populations, such as persons positive for HIV, to benefit from vaccination (Cooper et al., 2005). There are also promising results from studies into the adjuvant activity of CpG ODN for tumor vaccination. CD8+ T cells play a major role in effective tumor vaccination, and strong peptide specific T cell responses have been reported when patients have been vaccinated with a combination of CpG ODN and antigen (Speiser et al., 2005; Cornet et al., 2006).

Because TLR9 stimulation by CpG ODN activates the Th1 response, it could be reasoned that the induction of this Th1 response should influence the progress of diseases that present with a dominant Th2 pattern, such as that found in asthma and allergy. Indeed, the use of CpG ODN has been successfully used as an adjuvant in combination with a portion of the ragweed allergen, demonstrating how TLR9 ligation can specifically redirect the allergic Th2 response toward a nonallergic Th1 response (Simons et al., 2004). Although such CpG ODN-allergy combination vaccines have huge therapeutic potential, they can provide only allergen-specific redirection of allergic responses. The main goal remains the development of an inhaled CpG ODN therapy that could treat or prevent allergic airway responses in a broad spectrum of patients with allergies.

E. Inhibiting Toll-Like Receptors 7, 8, and 9

Apart from having potential as a target for adjuvants, TLRs 7, 8, and 9 have been strongly implicated in autoantibody production (Leadbetter et al., 2002; Lau et al., 2005). Immune complexes containing self-DNA and or self-RNA in mouse models of SLE have been shown to activate self-IgG–specific B-cells as a result of costimulation through TLR 7, 8, or 9 and the B-cell receptor. Both DNA immune complexes and RNA immune complexes from patients with SLE result in a Th1 pattern of cytokine production. It is probable that TLRs 7, 8, and 9 are activated by products of damaged tissue and provide the stimulus that gives rise to autoreactive B-cell proliferation, autoantibody production, and the subsequent development of autoimmune disease (Lau et al., 2005). It has long been known that patients with SLE have increased serum levels of IFNα, and recent studies have demonstrated that this increase correlates with disease activity. In fact, TLRs 7, 8, and 9 may play a dual role in SLE, because it has been shown that pDCs are the probable source of this IFNα, and the ligation of circulating RNA- or DNA-containing immune complexes with TLRs 7, 8, and 9 on the pDCs induce this IFNα expression (Vallin et al., 1999).

However, the role of TLR9 in SLE remains incomplete. Since the initial report that TLR9 was required for autoantibody formation in vivo, a number of studies have reported that loss of TLR9 in models of SLE actually aggravates the disease, suggesting a possible regulatory role for constitutive TLR9 expression in autoimmune disease (Christensen et al., 2005; Bauer et al., 2008). A more prominent role has been demonstrated for TLR7 in the pathogenesis of SLE, with recent reports demonstrating that the expression levels of TLR7 alone can influence autoimmune disease (Pisitkun et al., 2006). Pisitkun et al. (2006) tracked SLE susceptibility in mice to a Y-chromosome linked “autoimmune accelerator” locus termed Yaa. Yaa is not due to a mutation in a normal Y chromosome gene; instead, it is due to a duplication of a segment of X chromosomal DNA that has been transposed onto the Y chromosome. This effectively doubles the TLR7 gene dosage and responsiveness of B cells to TLR7 ligands, redirecting the disease from DNA- to RNA-associated pattern of autoantibodies. These results demonstrate that merely doubling the level of TLR7 expression can induce autoimmunity. Furthermore, it was reported that SLE-prone mice deficient in TLR7 showed reduced severity of autoimmune disease and that antagonism of TLR7 prevented autoimmune lung and kidney injury (Christensen et al., 2006; Pawar et al., 2007).

Overall these studies into SLE imply that a TLR9 and/or TLR7 antagonist would be predicted to have dual therapeutic benefits by inhibiting the major source of IFNα from pDCs and by inhibiting the costimulation and therefore activation of RNA/DNA immune complex-specific B cells, with the consequential inhibition of autoantibody production. In fact, chloroquine and its related compounds have been used since the 1950s to treat SLE, and their mechanism of action is now known to be inhibition of TLRs 7, 8, and 9, probably by blocking their ability to signal by increasing the pH of the endo-/lysosomes where they reside (Kalia and Dutz, 2007). Several types of inhibitory or suppressive ODNs have been described recently, some of which have been shown to block IFNα and reduce symptoms in SLE murine models, therefore representing a promising therapeutic agent for treatment of SLE (Dong et al., 2005; Barrat et al., 2007).

A point to take account of when considering TLR7/TLR8/TLR9 agonists as therapeutic agents is the potential for the induction of autoimmune disease. Because TLR7/TLR8/TLR9 agonists induce the secretion of IFNα and activate a Th1-weighted immune response, a hallmark feature of autoimmune diseases, there is the possibility that their use may result in the induction of autoimmunity. However, CpG ODN has been given to millions of people in the form of Bacillus Calmette-Guérin, and there have been no reported cases of auto-
immunity to date (Krieg and Vollmer, 2007). If this trend proves to be extrapolated to the activation of the other endosomal TLRs (i.e., 3, 7, and 8), then the therapeutic potential for TLR agonists is practically unlimited and may allow for the targeting of a broad range of disease, including cancers, asthma, allergies, and infections.

VII. Future Considerations in Therapeutic Development

Table 1 provides a summary of the major diseases in which TLRs have been implicated. There are clearly many options for the targeting of TLRs, because the key function of TLRs is to induce cytokines, which are well validated in these diseases, and indeed successfully being targeted in the clinic. Why would TLRs represent a better set of targets than cytokines or other downstream processes in inflammation? They occur early in these pathways and so inhibiting them might be more potent. They may be, in fact, at the initiation of pathologic conditions, because it is possible that the conditions listed are triggered by infections, with the ensuing inflammation leading to the production of endogenous ligands for TLRs, which further propagate the inflammation. With a genetic background in which TLRs are overactive (or their inhibitors are underactive), this might pivot into chronicity, and so targeting TLRs might therefore lead to remission from this chronic inflammation.

In terms of how to target them, different approaches are being taken. Neutralizing antibodies to TLRs might be possible, but only for those on the cell surface, such as TLR2, TLR4, and TLR5. Small-molecule antagonists (e.g., eritoran against TLR4 or ODN-based inhibitors of TLR7) might be a better prospect, although these are not traditional “drug-like” molecules, and so it is hard to predict off-target effects and efficacy. Because there are kinases on the signaling pathways, these might also be amenable to inhibition. One concern here, however, is that such inhibitors might block multiple TLRs and therefore give rise to unwanted immunosuppression. Monotherapies against a specific TLR might not have this problem; based on knockout mouse studies, there seem to be less redundancy in TLRs in relation to inflammation, which may not be the case with infection, where multiple TLRs can engage with a given pathogen.

Adjuvancy may continue to yield new agents. Imiquimod is already approved for its antiviral effects, whereas MPL is approved as a vaccine adjuvant. We can expect further adjuvants to emerge that might improve vaccine efficacy or have antitumor effects. In terms of antagonism, we have data beyond phase II for only one TLR inhibitor—eritoran. As described in section II.D, its effects were significant but somewhat marginal. We therefore eagerly await trials with other TLR inhibitors, possibly in combination in the case of sepsis. In which indication might we see the first agent? SLE is of considerable interest here, because TLR7 has been implicated in disease pathogenesis and an antagonist might prove very useful.

<table>
<thead>
<tr>
<th>TLR</th>
<th>Tissue</th>
<th>Therapeutic Potential/Use</th>
<th>Action</th>
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<tbody>
<tr>
<td>TLR2</td>
<td>Heart/vasculature</td>
<td>Atherosclerosis</td>
<td>Antagonistic</td>
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<td></td>
<td>Lung</td>
<td>Asthma</td>
<td>Antagonistic</td>
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<td></td>
<td>Kidney</td>
<td>Ischemia/reperfusion</td>
<td>Antagonistic</td>
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<td>Pancreas</td>
<td>Diabetes</td>
<td>Antagonistic</td>
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<td>TLR3</td>
<td>Systemic</td>
<td>Vaccine adjuvant</td>
<td>Agonistic</td>
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<td></td>
<td>Breast</td>
<td>Anti-cancer</td>
<td>Agonistic</td>
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<td></td>
<td>Renal system</td>
<td>Anti-cancer</td>
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<td></td>
<td>Eye</td>
<td>CNV inhibition in AMD</td>
<td>Agonistic</td>
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<tr>
<td>TLR4</td>
<td>Heart/vasculature</td>
<td>Atherosclerosis</td>
<td>Antagonistic</td>
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<td></td>
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<td>Brain</td>
<td>Alzheimer's disease, Parkinson's disease</td>
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<td></td>
<td>Sepsis</td>
<td>Vaccine adjuvant</td>
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<tr>
<td>TLR5</td>
<td>Hematopoietic system and GI tract</td>
<td>Vaccine-adjuvant (influenza virus)</td>
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<td></td>
<td>Systemic</td>
<td>Treatment of Crohn’s disease</td>
<td>Antagonistic</td>
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<td></td>
<td>GI tract</td>
<td>Treatment of viral induced lesions (caused by papilloma virus and Herpes simplex virus)</td>
<td>Agonistic</td>
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<tr>
<td>TLR7/8</td>
<td>Skin</td>
<td>Treatment of viral induced lesions (caused by papilloma virus and Herpes simplex virus)</td>
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<td>Primary tumors</td>
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<td>Cutaneous metastases</td>
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<td>Asthma/allergy</td>
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We have come a long way from the discovery of the first Toll in the fruit fly. The intense interest around TLRs, shared by immunologists, biomedical researchers, and pharmacologists, should surely yield better and more targeted therapies for major pathologic conditions.

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