The Emerging Role of Toll-Like Receptor Pathways in Surgical Diseases

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Objective: To outline the emerging significance of Toll-like receptor (TLR) signaling pathways in surgical diseases.

Data Sources: A systematic review of the literature was undertaken by searching the MEDLINE database for the period 1966 to 2005 without language restriction.

Study Selection: Original or review articles that described experimental data on the activation of TLR signaling pathways in surgically relevant diseases were selected for inclusion in this review.

Data Extraction: Data were obtained from peer-reviewed articles and references.

Data Synthesis: The role of TLRs in the recognition of pathogens renders them a key figure in the activation of both innate and adaptive immune responses during sepsis. However, emerging evidence points to fundamentally important roles in ulcerative colitis, Crohn disease, and Helicobacter pylori infection in the gastrointestinal tract and in the development of atherosclerotic plaques in the cardiovascular system. Furthermore, recent studies suggest that the regulation of the TLR pathway fulfills a central role in anticancer immunotherapy and in organ rejection after transplantation.

Conclusion: Given the clinical significance of TLR pathways, the targeting of individual molecular components is likely to offer a broad range of future therapeutic modalities.

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TOLL-LIKE RECEPTORS (TLRs) belong to the pattern recognition receptor superfamily that recognizes distinct pathogen-associated molecular patterns. They have a broad range of functions, from being responsible for dorsoventral polarity in the Drosophila embryo to protecting the latter from fungal infections. Since 1997, 11 members of the mammalian homologue of the Drosophila Toll receptor family have been described.

Structurally, TLRs consist of a leucine-rich repeat in the extracellular domain and a Toll–interleukin 1 (IL-1) receptor homologous region (TIR domain) in their intracellular portion. TLR4 (the so-called endotoxin receptor) recognizes lipopolysaccharide (LPS) present in the cell wall of gram-negative bacteria and also endogenous ligands such as heat shock proteins (HSPs) 60 and 70. TLR2 is responsible for the recognition of peptidoglycans (PGN), lipoteichoic acid (LTA), and bacterial lipoprotein (BLP), as well as zymosan from fungi. TLR3 recognizes double-stranded RNA (dsRNA) from viruses. TLR5 is responsible for bacterial flagellin recognition. TLR9 is important in the recognition of unmethylated CpG DNA derived from bacteria. TLR1, TLR6, and TLR10 can coassociate with TLR2 and subsequently enhance ligand specificity.

Toll-like receptors represent an important link between the “innate” and “adaptive” immune systems. Activation of TLRs triggers nonspecific immune responses in monocytes and macrophages. In addition, TLRs are also found on immature dendritic cells (DCs) and are capable of triggering their maturation. In this manner, DCs matured through TLR activation stimulate specific or adaptive immune responses.

In this article, we review recent data that link various surgical diseases to TLR pathways. We searched the MEDLINE database for the period 1966 to 2005 without language restriction. Original or review articles that described experimental data published in original papers or summarized in review papers on the activation of TLR signaling pathways in surgically relevant diseases were selected for inclusion in this review.
A central role has been attributed to TIR domain–containing adaptor proteins (TIRAPs): TIRAP/MyD88-adaptor–like (Mal) and Toll/IL-1 receptor–domain-containing adaptor–inducing interferon β (TRIF) (Figure 1). This suggestion is based on the finding that a dominant negative form of TIRAP/Mal prevents TLR4- but not TLR9-mediated NF-κB activation. Yamamoto et al\(^{13}\) showed that mice deficient in both MyD88 and TRIF exhibit a complete loss of TLR4-mediated NF-κB activation. These data indicate that LPS-triggered NF-κB activation involves either MyD88 or TRIF. These molecules may represent future therapeutic targets in the regulation of TLR-mediated signaling.

**THE SIGNALING PATHWAYS OF TLRs**

To better understand the functions of the TLRs, their associated signaling pathways should first be described (Figure 1). TLR4 is the most widely studied TLR. After LPS ligation, TLR4 forms stable clusters (receptorsomes) that recruit the adaptor molecule, MD-2\(^2\) (Figure 1). MD-2–deficient mice are resistant to LPS-induced shock, as are TLR4-mutant and TLR4-deficient mice.\(^3\) During gram-negative septic shock, circulating LPS is bound by LPS-binding protein (LBP) in the serum. This complex is subsequently recognized by CD14, which facilitates LPS-induced signaling.\(^6\) MD-2 either resides in a complex with TLR4 or is secreted in the serum when in excess.\(^7\) Having ligated with TLR4 at the cell surface, LPS is internalized rapidly via an unknown mechanism.

TLR activation induces either a core TLR response or a specific cellular response via the induction of different signaling pathways. As part of the core signaling pathway, myeloid differentiation factor 88 (MyD88) plays a central role (Figure 1). MyD88 interacts with TLRs via their TIR domain. MyD88-deficient mice are unresponsive to LPS, and MyD88-deficient macrophages do not respond to PGN and lipoproteins (TLR2 ligand) or CpG DNA (TLR9 ligand). On the other hand, MyD88-deficient mice are highly susceptible to *Staphylococcus aureus* infection.\(^6\) MyD88 deficiency modulates the kinetics of the LPS-induced inflammatory response. The activation of nuclear regulatory factor κB (NF-κB) and c-Jun N-terminal kinases is delayed.\(^10\) Given the central role of this molecule in LPS-mediated signal transduction, it represents a potential focal point for future therapies.

**TLRs IN SEPSIS AND THE SYSTEMIC INFLAMMATORY RESPONSE SYNDROME**

Figure 2 summarizes surgical diseases that are currently associated with TLR pathways. Much of the evidence that supports a role for TLR-mediated signaling in sepsis stems from murine studies. TLR4 activation is now known to occur during the systemic inflammatory response syndrome (SIRS). The development of SIRS, after the administration of soluble heparan sulfate or elastase, is TLR4 dependent. In keeping with this observation, Johnson et al\(^{15}\) demonstrated that exogenous macromolecules acting on TLRs can initiate a process that culminates in SIRS.
In humans, up-regulation of TLR4 and TLR2 levels occurs in monocytes isolated from patients with sepsis. Takeuchi et al showed that in murine models of acute polymicrobial sepsis, TLR2, TLR4 messenger (mRNA), and TLR4 protein levels are up-regulated early during polymicrobial sepsis, which correlates with increased mortality. Transgenic mice that overexpress TLR4 are more sensitive to LPS. Absence of the TLR4 gene protects against LPS-induced lethality but makes the host more susceptible to gram-negative bacterial infection. TLR2 and MyD88 knockout mice show increased susceptibility to S. aureus infection compared with wild-type mice. In addition, MyD88-deficient mice are more resistant to polymicrobial sepsis. This finding points to a common TLR4/TLR2 signaling pathway that follows the activation of both receptors by gram-positive and gram-negative bacteria. Interestingly, TLR4, TLR2, and combined TLR4/TLR2 deficiency do not improve survival in experimental polymicrobial sepsis.

Several studies point to the importance of genetic alterations in influencing outcome in sepsis. Polymorphisms in the TLR4 gene have been implicated in the development of septic shock. Arbour et al showed that common, cosegregating missense mutations (Asp299Gly and Thr399Ile) in the TLR4 gene lead to a blunted inflammatory response after LPS inhalation in humans. Gram-negative infection occurs in a significantly higher percentage of the patients with allelic mutations. The Asp299Gly mutation was found to be associated with increased mortality in SIRS. In contrast, monocytes separated from heterozygous TLR4 mutation carriers did not demonstrate an altered response to LPS. However, a coding mutation in the MD-2 gene (TLR4 coreceptor) results in reduced LPS-induced signaling.

Polymorphisms in the TLR2 gene have been shown to correlate with both the incidence and outcome of septic shock. In vitro, functional studies confirmed that cells that express mutant TLR2 are significantly less responsive to TLR2 ligands than wild-type controls. In accordance with this, Meng et al demonstrated that an anti-TLR2 antibody prevents a lethal shocklike syndrome in mice.

The clinical significance of genetic alterations in the downstream pathways has been illustrated by Picard et al, who recently described the cases of 3 unrelated children with recurrent severe pyogenic bacterial infections. They identified a defect in human TLR-IRAK signaling in these children that resulted in an increased susceptibility to pyogenic bacteria. However, this defect was associated with a relative protection against most other ubiquitous pathogens (Figure 2).

The ability to predict a patient’s individual susceptibility to infection would represent an enormously powerful therapeutic advance. Given our emerging understanding of single-nucleotide polymorphisms and given microarray technology, it is foreseeable that a single test, performed before patients undergo surgery, would readily determine susceptibility to infection and thereby guide prophylactic treatments.

Tolerance to bacterial cell wall components represents an adaptive host response during infection. Tolerance may also lead to severe immunosuppression during sepsis. The role of the TLR pathway in the generation of tolerance is somewhat controversial. We and others have demonstrated that the phenomena of homotolerance and heterotolerance occur for ligands of TLR2, TLR4, TLR5, TLR7, and TLR9. Numerous intracellular mechanisms have been implicated in tolerance. Our laboratory has recently demonstrated a down-regulation of TLR2 surface expression in tolerance induced by exposure to low-dose bacterial lipoprotein. Other studies demonstrate reduced recruitment of MyD88 to TLR4 in LPS-induced tolerance. Emerging mechanisms include the down-regulation of IRAK-1 and IRAK-M as well as reduced colocalization between IRAK isoforms and MyD88.

Recently it was observed that TLR4 and MD-2 exist in a soluble form in the circulation. Soluble TLR4 attenuates LPS-induced NF-κB activation. Pugin et al demonstrated that soluble MD-2 levels are increased in the plasma, urine, and lung edema fluid from patients with severe sepsis. We have shown that increased hepatic LPS sensitivity is associated with transcriptional up-regulation of MD-2 in a mouse model for fulminant hepatic failure. Similarly, excessive TLR4 reactivity, which can occur as a “second hit” after severe injury, might lead to multiple organ failure.

Finally, the high-mobility group box 1 (HMGB1) protein has been shown to bind TLR2 and TLR4. Secreted HMGB1 acts as a cytokine, and therapeutic administration of HMGB1 antagonists rescues mice from lethal sepsis. Therefore, the interaction of HMGB1 with TLR2 and TLR4 may provide an explanation for the ability of HMGB1 to generate inflammatory responses and accelerate lethality in sepsis.

**TLRs IN THE GASTROINTESTINAL TRACT**

In normal conditions, intestinal epithelia strongly express TLR3 and TLR5, whereas TLR4 and TLR2 expression is weak. Rakoff-Nahoum et al demonstrated that the interaction between commensal bacteria and TLRs on surface epithelia is crucial to the maintenance of gut epithelial homeostasis and protection against gut injury.

In ulcerative colitis (UC) and Crohn disease (CD), TLR4 expression in primary epithelial cells is increased. In contrast, TLR3 expression is down-regulated in active UC but not in active CD. Interestingly, TLR2 and TLR5 levels remain unchanged during both. Recent findings indicate that in murine models of experimental colitis, TLR4/MD-2 and CD14 are expressed mainly in distally located colonic epithelial cells, whereas TLR2 is expressed primarily in the proximal colon. This is referred to as TLR compartmentalization in colitis.

Nucleotide-binding and oligomerization domain 2 (Nod2), a receptor that is structurally similar to TLRs, is also involved in intracellular LPS recognition. Mutations in the Nod2, TLR4 (Asp299Gly), and TLR9 genes are associated with increased susceptibility to CD. The Asp299Gly and Thr399Ile TLR4 mutations are associated with a higher incidence of UC.

Lodes et al showed that the TLR3 agonist, flagellin, is a dominant antigen in CD. Although TLR5 is localized mainly on the basolateral surface of colonic cell lines,
it occurs mainly at the apical pole of ileal enterocytes. TLR5 expression on intestinal endothelial cells (in colonic microvessels) raises the exciting prospect of an overlap between localized innate immune responses and systemic vascular responses.60

Several studies point to a role for TLRs in the recognition of Helicobacter pylori, a crucial pathogenic factor in chronic gastritis, peptic ulcer disease, and gastric neoplasia. TLR4 mRNA levels are up-regulated by H pylori in gastric epithelial cell lines.61 However, H pylori–activated cytokine production occurs independently of TLR4 activation.62 In addition, H pylori–associated flagellin evades TLR5, a property that may contribute to its persistence in the host63 (Figure 2).

**TLRs IN VASCULAR AND CARDIAC SURGERY**

Chlamydia pneumoniae infection is associated with the development of atherosclerotic plaques.64 Elevated C pneumoniae–derived HSP-60 levels are found in patients with peripheral vascular diseases and in experimental models of atherosclerosis. Elevated serum HSP-60 levels are found in patients with atherosclerosis and even in those with borderline hypertension. Numerous studies65,66 have identified the presence of C pneumoniae in the walls of abdominal aortic aneurysms. These findings point to an association between HSP-60 and early cardiovascular disease.67,68

TLR4 recognizes both the whole bacterium and C pneumoniae–derived HSP-60. Chlamydia pneumoniae induces proliferation of human vascular smooth muscle cells. This effect occurs via TLR4-mediated activation of p44/p42 MAPK.68 Furthermore, TLR4 levels are up-regulated in atherosclerotic plaques compared with the normal vessel wall.70 TLR4 polymorphisms have also been associated with the development of atherosclerotic lesions. Evidence is emerging that TLR4 polymorphisms are associated with a protective antiatherosclerotic effect in the clinical setting. Kiechl et al67 demonstrated that the Asp299Gly TLR4 allele is associated with a lower risk of carotid atherosclerosis and a smaller intima media thickness in the common carotid artery, either in the homozigous or heterozygous form. Furthermore, TLR4 polymorphisms are associated with a reduced risk of acute coronary events.72 However, TLR4 polymorphisms do not appear to correlate with a reduced risk of stroke.73 TLR2 has recently been implicated in carotid artery plaques and in particular with the vulnerable plaque phenotype.74 Hence, these findings indicate that the adaptive and/or innate immune response is implicated in atherogenesis and may therefore be modulated with a view to altering the disease (Figure 2).

Frantz et al75 showed increased TLR4 expression on the border of necrotic and viable myocardium. Increased TLR2 and TLR4 levels have been demonstrated in circulating monocytes 2 days after coronary artery bypass grafting.76 In contrast, a protective role has been shown for TLR2. TLR2 has been proved to attenuate the effects of oxidative stress–related NF-kB activation in the myocardium.77 In addition, TLR2 plays an important role in ventricular remodeling after myocardial infarction.78

**TLRs IN SURGICAL ONCOLOGY**

At present, many forms of immunotherapy exploit a tumor antigen–specific immune response that is initiated by antigen-presenting DCs. Numerous phase 1 and 2 trials are currently under way using autologous DC vaccines against non–small cell lung cancer, melanoma, hepatocellular carcinoma, and gastric, colon, breast, pancreas, prostate, ovarian, and cervical cancer.79 Toll-like receptors play a critical role in DC maturation. In many DC-based vaccines, maturation most likely results from the activation of TLRs by bacillus Calmette-Guérin (BCG) cell wall skeleton, dsRNA, LPS, PGN, lipoprotein, and CpG DNA.80,81 One of the best known adjuvants, BCG cell wall skeleton, is known to induce both TLR2 and TLR4.80 Imiquimod, a synthetic antitumor agent, is a TLR7 ligand. Synergistic activation of TLR3 and TLR9 resulted in an enhanced antitumor effect, as demonstrated by Whitmore et al82 (Figure 2). Toll-like receptor signaling also critically overlaps with apoptotic cascades. The regulation of apoptosis by TLR ligands represents an attractive anticancer treatment modality. TLR2 activation induces apoptosis in macrophages and endothelial cells by recruiting MyD88, IRAK-1, and the Fas-associated death domain (FADD)–caspase 8 (and hence the extrinsic) proapoptotic pathway.83,84

There is increasing evidence, albeit indirect, that implicates LPS (and therefore TLR) signaling in the accelerated growth of minimal residual neoplastic disease after excisional surgery for cancer cure. It is well accepted that after surgical stress, local and systemic neoplastic disease is potentiated and undergoes acceleration in growth.85 Although multiple mechanisms have been implicated in this phenomenon, enhanced bacterial translocation and systemic endotoxemia are directly correlated.86 We have demonstrated that antiendoxin agents, such as taurouidine and probiotics, exert a protective effect against accelerated metastatic tumor growth after surgery.87,88 Overall, these findings strongly implicate LPS signaling, and by definition TLR signaling, in the acceleration of metastatic tumor growth that follows excisional surgery for apparent cancer cure.

**TLRs IN TRANSPLANTATION**

Dendritic cells play a role in graft rejection after transplantation. The role of TLR signaling has been investigated in this regard. Hemmi et al89 showed that minor antigen–mismatched acute rejection does not occur in MyD88-deficient mice. This experimental observation correlates with the presence of decreased numbers of mature DCs in draining lymph nodes. An investigation of the ischemia-reperfusion injury after liver transplantation revealed that TLR2, CD14, and LBP levels were increased within 6 to 12 hours after liver transplantation90 and also that TLR4 deficiency attenuated hepatic ischemia-reperfusion injury.91 These findings indicate that multiple components of the LPS signaling pathway are activated during ischemia-reperfusion injury after liver transplantation.92 In keeping with this, TLR4 polymorphisms are associated with decreased acute rejection after lung transplantation.93 Regarding heart transplanta-
tion, allograft endothelial dysfunction is associated with elevated TLR4 levels on peripheral blood monocytes. Endothelial dysfunction is an early indicator of chronic rejection after cardiac transplantation. These data demonstrate that TLR pathways contribute to organ rejection and ischemia-reperfusion injury after liver transplantation (Figure 2).

COMMENT

Toll-like receptors recognize specific patterns present in molecules within microbes and then regulate the activation of both innate and adaptive immunity. Currently it is unclear whether the net effect of TLR activation is beneficial or detrimental to the host. Although TLR4 deficiency increases susceptibility to gram-negative bacterial sepsis, it protects against endotoxin-induced sepsis. Similarly, TLR2-deficient mice are more susceptible to S. aureus infection than are their wild-type counterparts. In humans, TLR4 polymorphisms are associated with an increased incidence of gram-negative sepsis but inflammatory hyporesponsiveness to septic ligands and inhaled LPS. Similarly, patients with certain types of TLR2 polymorphisms are at increased risk of life-threatening staphylococcal infections. These data indicate that defective TLR signaling is detrimental to the host in bacterial infection. However, when bacterial cell wall components are used as ligands, defective TLR signaling appears protective. The whole bacterium theoretically might activate other, as yet unknown, pattern recognition receptors besides TLRs. These receptors may interfere with the TLR signaling process or trigger the production of different effector molecules, resulting in an altered cytokine environment.

The complexity of signaling pathways triggered by TLRs has been extensively characterized. Some of these studies have focused on the relationship between inflammatory and apoptotic pathways. Apoptotic pathways can be activated by death receptors of the TNF family such as Fas, TNF receptor 1, or the TNF-related apoptosis-inducing ligand (TRAIL) receptors. The apoptotic cascade is activated after the recruitment of the adaptor protein FADD. Interestingly, TLR2, TLR3, and TLR4 are also able to recruit FADD. TLR2 in association with activated TLR6 will recruit FADD, whereas TLR3 recruits FADD via TRIF. Bannerman et al demonstrated that TLRs simultaneously trigger proapoptotic (FADD) and antiapoptotic (NF-κB) signaling pathways. It is reasonable to speculate that after TLR activation, certain intracellular regulatory mechanisms may direct the cell either to undergo cell death or to persist and subsequently generate an inflammatory response. Understanding crosstalk between inflammatory and apoptotic pathways will greatly affect the treatment of septic and neoplastic diseases.

The recent increase in TLR research has identified novel associations with numerous disease processes. Although these associations will undoubtedly enhance our understanding of these diseases, they should also generate novel therapeutic targets. More important, the diverse role of TLR signaling pathways in numerous surgical diseases strongly points to an overlap in terms of pathogenesis. The suggestion therefore arises that conditions formerly associated with different molecular processes may involve common signaling pathways. This is an enticing prospect, since the therapeutic subversion of these pathways may greatly simplify the treatment of surgical disease. Such is the current level of understanding of the TLR signaling processes, and the components thereof, that it may soon be possible to therapeutically dissect these pathways by selectively targeting adaptor molecules (Mal/TIRAP, TRIF, TRAM, and MyD88) or co-receptors (MD-2 and CD14). We suggest that the therapeutic manipulation of TLR signaling will ultimately find its place at the bedside and represent a powerful adjuvant in the management of surgical disease.

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