Cell. Mol. Life Sci. 63 (2006) 2901–2907
1420-682X/06/242901-7
DOI 10.1007/s00018-006-6189-1

Cellular and Molecular Life Sciences

Review

Mammalian toll-like receptors: from endogenous ligands to tissue regeneration

Z. Zhang* and H. J. Schluesener

Institute of Brain Research, University of Tuebingen, Calwer Str. 3, 72076 Tuebingen (Germany),
Fax: +49 7071 294846, e-mail: zhangzhiren@yahoo.com

Received 26 April 2006; received after revision 16 June 2006; accepted 24 August 2006
Online First 28 October 2006

Abstract. Following injury a complex but well-orchestrated cellular response stimulating wound healing and tissue regeneration is induced. The balance of different cytokines, growth factors and cells is important in regulating tissue reorganisation. The immune system is critically involved in this process. Toll-like receptors (TLRs) are essential to the innate immune system, recognising microbial pathogens. The recent identification of endogenous ligands of TLRs suggests that they function not only to induce defensive antimicrobial immune responses but also as a sensitive detection system to initiate tissue regeneration after injury. Here we present an overview of TLRs and their endogenous ligands, and also review the roles of TLRs in inducing tissue regeneration after injury and in maintaining homeostasis. The identification of endogenous TLR ligands and their involvement in inducing tissue regeneration will provide new options to improve tissue reorganization after injury.

Keywords. Toll-like receptor, endogenous ligand, tissue repair, mammal, intestinal mucosal homeostasis.

Introduction

Regeneration is a process that restores cells, tissues and structures lost or damaged by disease, injury or ageing. The human body has an inherent but limited ability to repair organs and tissues after damage. Regeneration is a complex process requiring the coordinated interaction between stem/progenitor cells, growth factors, cytokines, inflammatory components, vascular components and the extracellular matrix. The complexity of regeneration requires a well-orchestrated system to control this process. The evidence increasingly suggests the importance of immune mechanisms as part of this regulatory cascade. The innate immune response that serves to eliminate infections is probably also active in restoring the structural and functional integrity of injured organs [1–4].

The innate immune system employs pattern recognition receptors (PRRs) to identify the presence of infection by detecting structures that are unique to microbes [5, 6]. Toll-like receptors (TLRs) are an emerging family of PRRs that recognise pathogen-associated molecular patterns (PAMPs) and regulate the activation of both innate and adaptive immunity. To date, 12 TLRs have been identified in mice and all have been shown to play important roles in triggering defensive antimicrobial immune responses [7, 8]. Interestingly, accumulating evidence also suggests that the TLRs play a role in regeneration. Indeed, a deficiency in certain TLRs has been shown to impair liver and lung regeneration [9–12]. Furthermore, peripheral activation of certain TLRs induces cell proliferation, including neuronal progenitor cells, in the rat central nervous system (CNS) [13]. Therefore, the modulation of TLRs may be a potential therapeutic strategy to regulate organ regeneration. Here, mammalian TLRs will be introduced and the relationship between TLRs and regeneration in several organs will be reviewed.

* Corresponding author.

TLRs in mammals

TLRs constitute a phylogenetically conserved family of PRRs that recognise and discriminate a diverse array of microbial antigens. At least 12 TLRs have been identified in mammals, and they detect a remarkably diverse array of bacterial, viral and fungal molecular patters. TLRs are type I transmembrane receptors and are characterised by three structural features: a divergent ligand-binding extracellular domain with leucine-rich repeats, a short transmembrane domain and a highly homologous cytoplasmic toll/interleukin (IL-)1 receptor domain, which is essential for initiation of downstream signalling cascades [14]. Using their extracellular domains, TLRs recognise multiple PAMPs, like lipopolysaccharide (LPS, by TLR4) [15], lipopeptides and lipoproteins (by TLR2) [16], double-stranded viral RNA (by TLR3) [17], bacterial flagellin from both Gram-positive and Gram-negative bacteria (by TLR5) [18], the unmethylated cytosine-guanosine (CpG) dinucleotide DNA (by TLR9) [19] and single-stranded viral RNA (by TLR7/8) [20, 21].

In addition to their ligand specificity, functions of individual TLRs differ according to their expression pattern and signalling pathways. TLRs are differentially expressed by many distinct cell types, including monocytes/macrophages/microglia, dendritic cells, B cells, T cells, natural killer cells, mast cells, astrocytes, epithelial cells and a variety of endothelial cells [22]. According to cell type, ligand and many other factors, individual TLRs activate different signalling pathways via diverse cofactors and adaptor molecules triggering specific immune responses [23]. In humans at least five adaptor molecules have been identified: MyD88, Mal/TIRAP, TRIF/TICAM-1, TRAM/Tirp/TICAM-2 and SARM [24, 25]. The so-called classic pathway is mediated by MyD88 that leads to the translocation of the transcription factor NF-κB to the nucleus and subsequently results into transcription of genes of pro- and anti-inflammatory cytokines, chemokines and costimulatory molecules, which are involved in the elimination of pathogens, control of tissue homeostasis and also linkage to adaptive immunity. Activation of several other transcription factors, like AP-1, EIK-1, CREB and STATs, has also been observed in different TLR signalling pathways in mammal [7, 26, 27]. Differences in signalling pathways and associated biological responses are seen not only among different TLRs but also among the same TLR in different cell types and organs.

In addition to the recognition of conserved microbial products which signal the presence of an infection and trigger the cellular immune response, TLRs also detect some endogenous ligands that might signal other danger conditions, such as degradation products of macromolecules, products of proteolytic cascades, intracellular components of ruptured cells and products of genes that are activated by inflammation [28–30]. One of the most well-established endogenous ligands is the DNA-immunoglobulin complex (DNA-IC). The DNA-IC is a potent interferon-α stimulator and is known to play an important role in the pathogenesis of systemic lupus erythematosus [31]. Recent investigations have demonstrated that DNA-IC activates B cells and dendritic cells through TLR9 and the Fc gamma receptors IIa or III are required for their optimal activation [32–34]. Endogenous RNA, released from or associated with necrotic cells, which stimulate dendritic cells leading to interferon-α secretion, is the endogenous ligand of TLR3 [35]. TLR3, -7, -8 and -9 recognise nucleic acids but the discrimination between nucleic acids of mammalian versus microbial origin by these TLRs needs further investigation. Interestingly, these TLRs are localised intracellularly [36–39] and recognise nucleic acids in late endosomes-lysosomes [20, 36, 40, 41]. Under certain conditions, such as tissue injury or deficiency in clearing apoptotic cells, host-derived nucleic acids may become available to these TLRs and serve as endogenous ligands.

Heat shock proteins (HSPs) function as molecular chaperones and have been identified as endogenous ligands of TLR2 or TLR4. HSP60, HSP70 and GP96 mobilise NF-κB, activate mitogen-activated protein kinase and induce dendritic cell maturation and cytokine synthesis through their interaction with TLR2 or TLR4 [42, 43].

Another large group of endogenous TLR ligands are extracellular matrix breakdown products, such as hyaluronan fragments, heparan sulphate, fibrinogen, fibronectin extra domain A, lung surfactant protein A and high-mobility group box 1 (HMGB1) proteins. These endogenous ligands are exposed during cellular injury and extracellular matrix remodelling and are recognised by TLR4 or TLR2 (only HMGB1). Hyaluronan fragments released after tissue injury activate dendritic and endothelial cells through TLR4 and result in nuclear translocation of NF-κB and cytokine secretion [44, 45]. Heparan sulphate was reported to induce dendritic cell maturation through TLR4 [46]. Fibrinogen has the ability to induce the production of chemokines from macrophages through TLR4 [47]. The fibronectin extra domain A is produced in response to tissue injury and activates TLRs to provoke expression of genes involved in the inflammatory response [48]. Similarly, induction of the activation of the NF-κB signalling pathway and up-regulation of cytokine synthesis by lung surfactant protein A are dependent on the functional TLR4 complex [49]. Furthermore, HMGB1 protein, originally described as a DNA-binding protein, can also be released extracellularly during an acute inflammatory responses and generate inflammatory responses through TLR 2 and TLR 4 [50].

Many endogenous ligands of TLRs have been reported, however, and apart from self-nucleic acids, endogenous ligands have been shown to trigger TLR2 or TLR4,

prompting a discussion that endogenous ligands might be contaminated by bacterial LPS (TLR4) or lipoprotein (TLR2) during preparation [28, 51, 52]. Recently, studies showed that LPS- free HSP70 and HSP60 did not induce activation of TLR2 and TLR4 [for a review see ref. 53]. It is therefore very important to ensure that biochemical preparations of endogenous ligands are free of contaminating, highly active exogenous TLR ligands.

Obviously, the known endogenous ligands of TLRs are either molecules released from damaged cells or extracellular matrix breakdown products. The identification of TLR endogenous ligands challenges the traditional view that major functions of TLRs are considered to distinguish self-non-self but supports the 'danger-hypothesis' proposed by Matzinger [54, 55], who suggested that the immune system has primarily evolved to recognise danger signals rather than non-self signals. Furthermore, the mechanisms of endogenous activation of mammalian TLRs may provide key insights for the understanding of multiple pathophysiological conditions following injury.

Mammalian TLRs and tissue regeneration

Tissue regeneration is a complex process covering a variety of cellular processes, such as inflammation, angiogenesis, extracellular matrix synthesis, reepithelisation and collagen deposition. A variety of cell types, extracellular matrix components, cytokines and other soluble factors are involved in the regenerative process [1–4]. During tissue injury/regeneration, numerous cells die by necrosis and release their intracellular content. Furthermore, extracellular matrix turnover causes the production of breakdown products. As described above, both the intracellular content and extracellular matrix breakdown products provide endogenous ligands of TLRs, which stimulate the innate immune system through TLRs.

Interestingly, necrotic cells – but not healthy or apoptotic cells – activate dendritic cells to up-regulate expression of certain costimulatory and MHC class II molecules [56]. Sauter et al. [57] reported that necrotic tumor cells induce maturation of immunostimulatory dendritic cells, which express high levels of CD83, lysosome-associated membrane glycoprotein and costimulatory molecules CD40 and CD86. Basu et al. [58] further demonstrated that HSPs released from necrotic cells stimulate macrophages to secrete cytokines, and induce expression of antigen-presenting and costimulatory molecules on dendritic cells through the highly conserved NF-κB pathway [58]. Investigations by Li et al. [59] further proved that necrotic cells, but not apoptotic cells, activate NF-κB in viable fibroblasts, macrophages or dendritic cells to induce the expression of inflammatory and tissue repair genes, which are dependent on TLR2 expression. The induced genes include neutrophil-specific chemokines (cytokine-induced neutrophil chemoattractant and macrophage inflammatory protein-2), metalloproteinase 3 and vascular endothelial growth factor, all of which play a role in tissue repair and remodelling. Thus, following injury, necrotic cells can act at least through TLR2 to recruit neutrophils and initiate the process of tissue repair.

Hyaluronan, one of the major structural components of the extracellular matrix, is a high-molecular-weight polymer [60] but undergoes rapid degradation at sites of tissue injury and inflammation thereby serving as an indicator of tissue injury [60–62]. The soluble low-molecular-weight degradation products of hyaluronan have been implicated in a variety of inflammatory and repair processes [63] and are known to signal through TLR4 in dendritic cells [45] and endothelial cells [44]. A low-molecular-weight fragment of hyaluronan induces the maturation and activation of human and murine dendritic cells via a TLR4-dependent pathway, which includes the phosphorylation of p38 and p42/p44 MAPK and the translocation of NF-κB to the nucleus [45]. Furthermore, Taylor et al. [44] demonstrated that hyaluronan fragments stimulate chemokine secretion by endothelial cells through TLR4 [44]. The effects and mechanisms of hyaluronan-TLR interaction were investigated in a lung injury model by Jiang and colleagues in knockout mice [9, 10]. They showed that hyaluronan fragments produced after acute lung injury stimulate macrophage chemokine production in a TLR4- and TLR2-dependent way. Furthermore, *Myd88*(–/–) and *Tlr4*(–/–)*Tlr2*(–/–) mice showed impaired transepithelial migration of neutrophils but decreased survival and enhanced epithelial cell apoptosis after lung injury. Protective effects of hyaluronan-TLR2 or -4 interactions are dependent on activation of NF-κB. Therefore, hyaluronan-TLR2 and hyaluronan-TLR4 interactions provide signals that initiate inflammatory responses, maintain epithelial cell integrity and promote recovery from acute lung injury. In addition, hyaluronan and TLR2/4 serve as a sensitive detection system for initiation of the wound defence and repair process.

The involvement of TLRs in liver regeneration after partial hepatectomy has been demonstrated recently [11, 12]. MyD88 is a common molecule required by most TLRs, except TLR3 [64, 65], to activate NF-κB, resulting in the production of a variety of cytokines, including IL-6 and tumor necrosis factor-α (TNF-α), which play a critical role in activation of the priming phase of liver regeneration [66–68]. In *MyD88* knockout mice, NF-κB activation, IL-6 and TNF-α were greatly reduced and early recovery of liver mass is impaired following partial hepatectomy [11, 12]. However, MyD88 was reported to mediate negative growth control, including growth suppression and apoptosis [69]. Thus, impaired regeneration in *MyD88* knockout mice might be due to other signalling pathways.

Endogenous ligands can signal through TLRs to induce tissue regeneration, and exogenous stimulators of TLRs also have been shown to contribute to tissue regeneration. Macrophage-activating lipopeptide-2 (MALP2) acts through a combination of TLR2 and TLR6 on macrophages and fibroblasts to induce chemokine expression [70, 71]. Application of MALP2 to skin wounds of diabetic mice stimulated the production of monocyte chemoattractant protein-1 at the wound site, which leads to increased macrophage infiltration, and stimulated wound healing in the initial inflammatory phase, triggering the natural process of wound repair and resulting in normal restructured skin without hypertrophic growth of granulation tissue [72]. Glezer et al. [73] studied the effects of LPS on CNS remyelination. Brain infusion of LPS activated microglia and induces the expression of platelet-derived growth factor-α. Intracerebral infusion of LPS significantly induced proliferation of oligodendrocyte progenitor cells and resulted in less accumulation of myelin debris and byproducts that may delay brain recovery following ethidium-bromide-induced brain damage. Furthermore, LPS-triggered recruitment of oligodendrocyte progenitor cells and accelerated remyelination after brain injury were observed in wild-type mice but not in mice bearing a missense mutation in the *TLR4* gene. Thus, under certain conditions, LPS can trigger tissue regeneration through TLR4 [73]. A study by Su et al. [13] investigated the effects of peripheral administration of TLR ligands on *in vivo* spinal cord cell proliferation. Bolus intraperitoneal injection of polyinosine-polycytidylic acid (TLR3 ligand), LPS or R848 (TLR7/8 ligand) temporarily increased cell proliferation in the rat spinal cord. Only a few of the proliferating cells were of microglial origin, but BrdU(+)/nestin(+) cells were found, suggestive of a proliferation of local progenitor cells. In addition, stimulation of cell proliferation correlated with activation of microglia. These findings suggest an intricate interaction of cellular processes of the innate immune system and regeneration. In rats or mice, local administration of LPS after spinal cord injury greatly improved locomotor function and glial-derived neurotrophic factor expression, suggesting that LPS-induced local inflammation leads to repair of CNS tissue [74, 75].

Therefore, following tissue injury, certain endogenous TLR ligands are released, which activate TLRs, resulting in expression of a variety of genes to initiate tissue regeneration. Chemokines, like cytokine-induced neutrophil chemoattractant and macrophage inflammatory protein-2, can induce infiltration of neutrophils and macrophages to remove necrotic cells. The increased expression of metalloproteinase may function to provoke extracellular matrix remodelling. Furthermore, the induction of certain cytokines, such as IL-6, TNF-α and endothelial growth factors, can initiate cell proliferation and angiogenesis. Thus, the activation of TLRs following injury promotes tissue regeneration through several mechanisms, some of which are summarized in Figure 1.

Mammalian TLRs and intestinal mucosal homeostasis

In addition to inducing tissue regeneration following injury, TLRs also play important roles in maintaining intestinal mucosal homeostasis through interaction with intestinal commensals and their products [for reviews see refs. 76–78]. In the gut, commensal-derived TLR ligands actively induce, via TLRs, expression of genes that func-

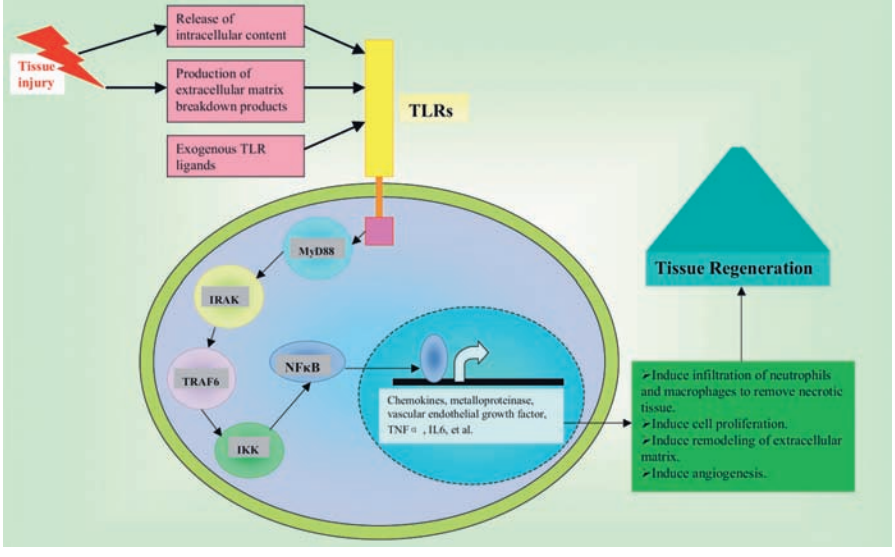

Figure 1. Schematic representation of potential mechanisms by which TLRs are involved in tissue regeneration following injury.

tion in cell differentiation or maturation and induce basal activation of TLRs, which facilitate rapid restitution and limited inflammatory responses [79, 80]. Furthermore, the interaction of commensals with TLRs strengthens intestinal epithelial barrier resistance [81–83]. Commensals can also activate TLRs to prevent allergic sensitisation to food antigen. *TLR4* knockout mice are highly susceptible to food antigen, with up-regulated TH2 cytokines, IL-4 and IL-13. LPS may activate through TLR4 to induce a TH1-type immune response to inhibit TH2-type inflammation [76, 84]. Interestingly, the activation of certain TLRs, such as TLR4, by commensals can induce the expression of proteins, like Tollip, PPARγ and TIR8, that negatively regulate TLR signalling pathways, resulting in suppression of inflammatory responses [77, 85, 86, 87]. Further administration of CpG-DNA ameliorates the severity of dextran-sodium-sulphate-induced colitis via TLR9 and limits cytokine-derived intestinal epithelial proinflammatory immune responses [78].

Concluding remarks

The crucial role played by TLRs in mediating innate immunity against microbial pathogens is well recognised. The concept of endogenous ligands for TLRs, like necrotic cells, HSPs and extracellular matrix breakdown products has become established over the last few years. These endogenous ligands are released following tissue injury or inflammation and activate TLRs to induce expression of various cytokines. These interesting findings suggest that TLRs are not only involved in the recognition of microbes, but also of endogenous harmful stimuli. Furthermore, the ability of certain TLR agonists to induce tissue regeneration has been demonstrated in several animal models and the interactions between commensals and TLRs are important for intestinal mucosal homeostasis. Although deteriorative effects of TLRs following tissue injury have also been reported [88, 89], the recognition of the tissue-protective effects of TLRs following injury provides a new insight into the functions of TLRs in tissue regeneration and suggestions for the development of therapeutic strategies to improve tissue repair.

1 Alessandri, G., Emanueli, C. and Madeddu, P. (2004) Genetically engineered stem cell therapy for tissue regeneration. Ann. N. Y. Acad. Sci. 1015, 271–284.
2 Frantz, S., Vincent, K. A., Feron, O. and Kelly, R. A. (2005) Innate immunity and angiogenesis. Circ. Res. 96, 15–26.
3 Martin, P. (1997) Wound healing – aiming for perfect skin regeneration. Science 276, 75–81.
4 Saltzman, W. M. (1999) Delivering tissue regeneration. Nat. Biotechnol. 17, 534–535.
5 Medzhitov, R. and Janeway, C. A. Jr. (1998) Innate immune recognition and control of adaptive immune responses. Semin. Immunol. 10, 351–353.
6 Medzhitov, R. and Janeway, C. A. Jr. (2002) Innate immune recognition. Annu. Rev. Immunol. 20, 197–216.
7 Akira, S. and Sato, S. (2003) Toll-like receptors and their signalling mechanisms. Scand. J. Infect. Dis. 35, 555–562.
8 Akira, S., Takeda, K. and Kaisho, T. (2001) Toll-like receptors: critical proteins linking innate and acquired immunity. Nat. Immunol. 2, 675–680.
9 Jiang, D., Liang, J., Fan, J., Yu, S., Chen, S., Luo, Y., Prestwich, G. D., Mascarenhas, M. M., Garg, H. G., Quinn, D. A., Homer, R. J., Goldstein, D. R., Bucala, R., Lee, P. J., Medzhitov, R. and Noble, P. W. (2005) Regulation of lung injury and repair by Toll-like receptors and hyaluronan. Nat. Med. 11, 1173–1179.
10 O'Neill, L. A. (2005) TLRs play good cop, bad cop in the lung. Nat. Med. 11, 1161–1162.
11 Campbell, J. S., Riehle, K. J., Brooling, J. T., Bauer, R. L., Mitchell, C. and Fausto, N. (2006) Proinflammatory cytokine production in liver regeneration is myd88-dependent, but independent of cd14, tlr2, and tlr4. J. Immunol. 176, 2522–2528.
12 Seki, E., Tsutsui, H., Iimuro, Y., Naka, T., Son, G., Akira, S., Kishimoto, T., Nakanishi, K. and Fujimoto, J. (2005) Contribution of Toll-like receptor/myeloid differentiation factor 88 signalling to murine liver regeneration. Hepatology 41, 443–450.
13 Su, Y., Zhang, Z., Trautmann, K., Xu, S. and Schluesener, H. J. (2005) TLR and NOD2 ligands induce cell proliferation in the rat intact spinal cord. J. Neuropathol. Exp. Neurol. 64, 991–997.
14 Aderem, A. and Ulevitch, R. J. (2000) Toll-like receptors in the induction of the innate immune response. Nature 406, 782–787.
15 Poltorak, A., He, X., Smirnova, I., Liu, M. Y., Van Huffel, C., Du, X., Birdwell, D., Alejos, E., Silva, M., Galanos, C., Freudenberg, M., Ricciardi-Castagnoli, P., Layton, B. and Beutler, B. (1998) Defective LPS signalling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. Science 282, 2085–2088.
16 Takeuchi, O., Hoshino, K., Kawai, T., Sanjo, H., Takada, H., Ogawa, T., Takeda, K. and Akira, S. (1999) Differential roles of TLR2 and TLR4 in recognition of gram-negative and gram-positive bacterial cell wall components. Immunity 11, 443–451.
17 Alexopoulou, L., Holt, A. C., Medzhitov, R. and Flavell, R. A. (2001) Recognition of double-stranded RNA and activation of NF-kappaB by Toll-like receptor 3. Nature 413, 732–738.
18 Hayashi, F., Smith, K. D., Ozinsky, A., Hawn, T. R., Yi, E. C., Goodlett, D. R., Eng, J. K., Akira, S., Underhill, D. M. and Aderem, A. (2001) The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. Nature 410, 1099–1103.
19 Hemmi, H., Takeuchi, O., Kawai, T., Kaisho, T., Sato, S., Sanjo, H., Matsumoto, M., Hoshino, K., Wagner, H., Takeda, K. and Akira, S. (2000) A Toll-like receptor recognizes bacterial DNA. Nature 408, 740–745.
20 Lund, J. M., Alexopoulou, L., Sato, A., Karow, M., Adams, N. C., Gale, N. W., Iwasaki, A. and Flavell, R. A. (2004) Recognition of single-stranded RNA viruses by Toll-like receptor 7. Proc. Natl. Acad. Sci. USA 101, 5598–5603.
21 Heil, F., Hemmi, H., Hochrein, H., Ampenberger, F., Kirschning, C., Akira, S., Lipford, G., Wagner, H. and Bauer, S. (2004) Species-specific recognition of single-stranded RNA via toll-like receptor 7 and 8. Science 303, 1526–1529.
22 Takeda, K., Kaisho, T. and Akira, S. (2003) Toll-like receptors. Annu. Rev. Immunol. 21, 335–376.
23 Iwasaki, A. and Medzhitov, R. (2004) Toll-like receptor control of the adaptive immune responses. Nat. Immunol. 5, 987–995.
24 O'Neill, L. A., Fitzgerald, K. A. and Bowie, A. G. (2003) The Toll-IL-1 receptor adaptor family grows to five members. Trends Immunol. 24, 286–290.
25 Yamamoto, M., Takeda, K. and Akira, S. (2004) TIR domain-containing adaptors define the specificity of TLR signalling. Mol. Immunol. 40, 861–868.
26 Andreakos, E., Foxwell, B. and Feldmann, M. (2004) Is targeting Toll-like receptors and their signalling pathway a useful therapeutic approach to modulating cytokine-driven inflammation? Immunol. Rev. 202, 250–265.

27 Vogel, S. N., Fitzgerald, K. A. and Fenton, M. J. (2003) TLRs: differential adapter utilization by toll-like receptors mediates TLR-specific patterns of gene expression. Mol. Interv. 3, 466–477.
28 Beg, A. A. (2002) Endogenous ligands of Toll-like receptors: implications for regulating inflammatory and immune responses. Trends Immunol. 23, 509–512.
29 Johnson, G. B., Brunn, G. J. and Platt, J. L. (2003) Activation of mammalian Toll-like receptors by endogenous agonists. Crit. Rev. Immunol. 23, 15–44.
30 Seong, S. Y. and Matzinger, P. (2004) Hydrophobicity: an ancient damage-associated molecular pattern that initiates innate immune responses. Nat. Rev. Immunol. 4, 469–478.
31 Barrat, F. J., Meeker, T., Gregorio, J., Chan, J. H., Uematsu, S., Akira, S., Chang, B., Duramad, O. and Coffman, R. L. (2005) Nucleic acids of mammalian origin can act as endogenous ligands for Toll-like receptors and may promote systemic lupus erythematosus. J. Exp. Med. 202, 1131–1139.
32 Boule, M. W., Broughton, C., Mackay, F., Akira, S., Marshak-Rothstein, A. and Rifkin, I. R. (2004) Toll-like receptor 9-dependent and -independent dendritic cell activation by chromatin-immunoglobulin G complexes. J. Exp. Med. 199, 1631–1640.
33 Leadbetter, E. A., Rifkin, I. R., Hohlbaum, A. M., Beaudette, B. C., Shlomchik, M. J. and Marshak-Rothstein, A. (2002) Chromatin-IgG complexes activate B cells by dual engagement of IgM and Toll-like receptors. Nature 416, 603–607.
34 Viglianti, G. A., Lau, C. M., Hanley, T. M., Miko, B. A., Shlomchik, M. J. and Marshak-Rothstein, A. (2003) Activation of autoreactive B cells by CpG dsDNA. Immunity 19, 837–847.
35 Kariko, K., Ni, H., Capodici, J., Lamphier, M. and Weissman, D. (2004) mRNA is an endogenous ligand for Toll-like receptor 3. J. Biol. Chem. 279, 12542–12550.
36 Latz, E., Schoenemeyer, A., Visintin, A., Fitzgerald, K. A., Monks, B. G., Knetter, C. F., Lien, E., Nilsen, N. J., Espevik, T. and Golenbock, D. T. (2004) TLR9 signals after translocating from the ER to CpG DNA in the lysosome. Nat. Immunol. 5, 190–198.
37 Heil, F., Ahmad-Nejad, P., Hemmi, H., Hochrein, H., Ampenberger, F., Gellert, T., Dietrich, H., Lipford, G., Takeda, K., Akira, S., Wagner, H. and Bauer, S. (2003) The Toll-like receptor 7 (TLR7)-specific stimulus loxoribine uncovers a strong relationship within the TLR7, 8 and 9 subfamily. Eur. J. Immunol. 33, 2987–2997.
38 Lee, J., Chuang, T. H., Redecke, V., She, L., Pitha, P. M., Carson, D. A., Raz, E. and Cottam, H. B. (2003) Molecular basis for the immunostimulatory activity of guanine nucleoside analogs: activation of Toll-like receptor 7. Proc. Natl. Acad. Sci. USA 100, 6646–6651.
39 Matsumoto, M., Funami, K., Tanabe, M., Oshiumi, H., Shingai, M., Seto, Y., Yamamoto, A. and Seya, T. (2003) Subcellular localization of Toll-like receptor 3 in human dendritic cells. J. Immunol. 171, 3154–3162.
40 Diebold, S. S., Kaisho, T., Hemmi, H., Akira, S. and Reis e Sousa, C. (2004) Innate antiviral responses by means of TLR7-mediated recognition of single-stranded RNA. Science 303, 1529–1531.
41 Lund, J., Sato, A., Akira, S., Medzhitov, R. and Iwasaki, A. (2003) Toll-like receptor 9-mediated recognition of Herpes simplex virus-2 by plasmacytoid dendritic cells. J. Exp. Med. 198, 513–520.
42 Liu, B., Dai, J., Zheng, H., Stoilova, D., Sun, S. and Li, Z. (2003) Cell surface expression of an endoplasmic reticulum resident heat shock protein gp96 triggers MyD88-dependent systemic autoimmune diseases. Proc. Natl. Acad. Sci. USA 100, 15824–15829.
43 Vabulas, R. M., Wagner, H. and Schild, H. (2002) Heat shock proteins as ligands of toll-like receptors. Curr. Top. Microbiol. Immunol. 270, 169–184.
44 Taylor, K. R., Trowbridge, J. M., Rudisill, J. A., Termeer, C. C., Simon, J. C. and Gallo, R. L. (2004) Hyaluronan fragments stimulate endothelial recognition of injury through TLR4. J. Biol. Chem. 279, 17079–17084.
45 Termeer, C., Benedix, F., Sleeman, J., Fieber, C., Voith, U., Ahrens, T., Miyake, K., Freudenberg, M., Galanos, C. and Simon, J. C. (2002) Oligosaccharides of Hyaluronan activate dendritic cells via toll-like receptor 4. J. Exp. Med. 195, 99–111.
46 Johnson, G. B., Brunn, G. J., Kodaira, Y. and Platt, J. L. (2002) Receptor-mediated monitoring of tissue well-being via detection of soluble heparan sulphate by Toll-like receptor 4. J. Immunol. 168, 5233–5239.
47 Smiley, S. T., King, J. A. and Hancock, W. W. (2001) Fibrinogen stimulates macrophage chemokine secretion through toll-like receptor 4. J. Immunol. 167, 2887–2894.
48 Okamura, Y., Watari, M., Jerud, E. S., Young, D. W., Ishizaka, S. T., Rose, J., Chow, J. C. and Strauss, J. F. 3rd. (2001) The extra domain A of fibronectin activates Toll-like receptor 4. J. Biol. Chem. 276, 10229–10233.
49 Guillot, L., Balloy, V., McCormack, F. X., Golenbock, D. T., Chignard, M. and Si-Tahar, M. (2002) Cutting edge: the immunostimulatory activity of the lung surfactant protein-A involves Toll-like receptor 4. J. Immunol. 168, 5989–5992.
50 Park, J. S., Svetkauskaite, D., He, Q., Kim, J. Y., Strassheim, D., Ishizaka, A. and Abraham, E. (2004) Involvement of toll-like receptors 2 and 4 in cellular activation by high mobility group box 1 protein. J. Biol. Chem. 279, 7370–7377.
51 Tsan, M. F. and Gao, B. (2004) Endogenous ligands of Toll-like receptors. J. Leukoc. Biol. 76, 514–519.
52 Akira, S. and Takeda, K. (2004) Toll-like receptor signalling. Nat. Rev. Immunol. 4, 499–511.
53 Tsan, M. F. and Gao, B. (2004) Heat shock protein and innate immunity. Cell Mol. Immunol. 1, 274–279.
54 Matzinger, P. (2002) The danger model: a renewed sense of self. Science 296, 301–305.
55 Matzinger, P. (1994) Tolerance, danger, and the extended family. Annu. Rev. Immunol. 12, 991–1045.
56 Gallucci, S., Lolkema, M. and Matzinger, P. (1999) Natural adjuvants: endogenous activators of dendritic cells. Nat. Med. 5, 1249–1255.
57 Sauter, B., Albert, M. L., Francisco, L., Larsson, M., Somersan, S. and Bhardwaj, N. (2000) Consequences of cell death: exposure to necrotic tumor cells, but not primary tissue cells or apoptotic cells, induces the maturation of immunostimulatory dendritic cells. J. Exp. Med. 191, 423–434.
58 Basu, S., Binder, R. J., Suto, R., Anderson, K. M. and Srivastava, P. K. (2000) Necrotic but not apoptotic cell death releases heat shock proteins, which deliver a partial maturation signal to dendritic cells and activate the NF-kappa B pathway. Int. Immunol. 12, 1539–1546.
59 Li, M., Carpio, D. F., Zheng, Y., Bruzzo, P., Singh, V., Ouaaz, F., Medzhitov, R. M. and Beg, A. A. (2001) An essential role of the NF-kappa B/Toll-like receptor pathway in induction of inflammatory and tissue repair gene expression by necrotic cells. J. Immunol. 166, 7128–7135.
60 Fraser, J. R., Laurent, T. C. and Laurent, U. B. (1997) Hyaluronan: its nature, distribution, functions and turnover. J. Intern. Med. 242, 27–33.
61 Agren, U. M., Tammi, R. H. and Tammi, M. I. (1997) Reactive oxygen species contribute to epidermal hyaluronan catabolism in human skin organ culture. Free Radic. Biol. Med. 23, 996–1001.
62 Teder, P., Vandivier, R. W., Jiang, D., Liang, J., Cohn, L., Pure, E., Henson, P. M. and Noble, P. W. (2002) Resolution of lung inflammation by CD44. Science 296, 155–158.
63 Termeer, C., Sleeman, J. P. and Simon, J. C. (2003) Hyaluronan-magic glue for the regulation of the immune response? Trends Immunol. 24, 112–114.

64 Yamamoto, M., Sato, S., Hemmi, H., Hoshino, K., Kaisho, T., Sanjo, H., Takeuchi, O., Sugiyama, M., Okabe, M., Takeda, K. and Akira, S. (2003) Role of adaptor TRIF in the MyD88-independent toll-like receptor signalling pathway. Science 301, 640–643.
65 Meylan, E., Burns, K., Hofmann, K., Blancheteau, V., Martinon, F., Kelliher, M. and Tschopp, J. (2004) RIP1 is an essential mediator of Toll-like receptor 3-induced NF-kappa B activation. Nat. Immunol. 5, 503–507.
66 Yamada, Y., Kirillova, I., Peschon, J. J. and Fausto, N. (1997) Initiation of liver growth by tumor necrosis factor: deficient liver regeneration in mice lacking type I tumor necrosis factor receptor. Proc. Natl. Acad. Sci. USA 94, 1441–1446.
67 Sakamoto, T., Liu, Z., Murase, N., Ezure, T., Yokomuro, S., Poli, V. and Demetris, A. J. (1999) Mitosis and apoptosis in the liver of interleukin-6-deficient mice after partial hepatectomy. Hepatology 29, 403–411.
68 Aldeguer, X., Debonera, F., Shaked, A., Krasinkas, A. M., Gelman, A. E., Que, X., Zamir, G. A., Hiroyasu, S., Kovalovich, K. K., Taub, R. and Olthoff, K. M. (2002) Interleukin-6 from intrahepatic cells of bone marrow origin is required for normal murine liver regeneration. Hepatology 35, 40–48.
69 Liebermann, D. A. and Hoffman, B. (1998) MyD genes in negative growth control. Oncogene 17, 3319–3329.
70 Takeuchi, O., Kawai, T., Muhlradt, P. F., Morr, M., Radolf, J. D., Zychlinsky, A., Takeda, K. and Akira, S. (2001) Discrimination of bacterial lipoproteins by Toll-like receptor 6. Int. Immunol. 13, 933–940.
71 Morr, M., Takeuchi, O., Akira, S., Simon, M. M. and Muhlradt, P. F. (2002) Differential recognition of structural details of bacterial lipopeptides by toll-like receptors. Eur. J. Immunol. 32, 3337–3347.
72 Deiters, U., Barsig, J., Tawil, B. and Muhlradt, P. F. (2004) The macrophage-activating lipopeptide-2 accelerates wound healing in diabetic mice. Exp. Dermatol. 13, 731–739.
73 Glezer, I., Lapointe, A. and Rivest, S. (2006) Innate immunity triggers oligodendrocyte progenitor reactivity and confines damages to brain injuries. FASEB J. 20,750–752.
74 Hashimoto, M., Ito, T., Fukumitsu, H., Nomoto, H., Furukawa, Y. and Furukawa, S. (2005) Stimulation of production of glial cell line-derived neurotrophic factor and nitric oxide by lipopolysaccharide with different dose-responsiveness in cultured rat macrophages. Biomed. Res. 26, 223–229.
75 Hashimoto, M., Nitta, A., Fukumitsu, H., Nomoto, H., Shen, L. and Furukawa, S. (2005) Inflammation-induced GDNF improves locomotor function after spinal cord injury. Neuroreport 16, 99–102.
76 Prioult, G. and Nagler-Anderson, C. (2005) Mucosal immunity and allergic responses: lack of regulation and/or lack of microbial stimulation? Immunol. Rev. 206, 204–218.
77 Cario, E. and Podolsky, D. K. (2005) Intestinal epithelial TOLLerance versus inTOLLerance of commensals. Mol. Immunol. 42, 887–893.
78 Cario, E. (2005) Bacterial interactions with cells of the intestinal mucosa: Toll-like receptors and NOD2. Gut 54, 1182–1193.
79 Rakoff-Nahoum, S., Paglino, J., Eslami-Varzaneh, F., Edberg, S. and Medzhitov, R. (2004) Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. Cell 118, 229–241.
80 Hooper, L. V., Wong, M. H., Thelin, A., Hansson, L., Falk, P. G. and Gordon, J. I. (2001) Molecular analysis of commensal host-microbial relationships in the intestine. Science 291, 881–884.
81 Cario, E., Gerken, G. and Podolsky, D. K. (2004) Toll-like receptor 2 enhances ZO-1-associated intestinal epithelial barrier integrity via protein kinase, C. Gastroenterology 127, 224–238.
82 Madsen, K., Cornish, A., Soper, P., McKaigney, C., Jijon, H., Yachimec, C., Doyle, J., Jewell, L. and De Simone, C. (2001) Probiotic bacteria enhance murine and human intestinal epithelial barrier function. Gastroenterology 121, 580–591.
83 Otte, J. M. and Podolsky, D. K. (2004) Functional modulation of enterocytes by gram-positive and gram-negative microorganisms. Am. J. Physiol. Gastrointest. Liver Physiol. 286, G613–626.
84 Bashir, M. E., Louie, S., Shi, H. N. and Nagler-Anderson, C. (2004) Toll-like receptor 4 signalling by intestinal microbes influences susceptibility to food allergy. J. Immunol. 172, 6978–6987.
85 Dubuquoy, L., Jansson, E. A., Deeb, S., Rakotobe, S., Karoui, M., Colombel, J. F., Auwerx, J., Pettersson, S. and Desreumaux, P. (2003) Impaired expression of peroxisome proliferator-activated receptor gamma in ulcerative colitis. Gastroenterology 124, 1265–1276.
86 Kelly, D., Campbell, J. I., King, T. P., Grant, G., Jansson, E. A., Coutts, A. G., Pettersson, S. and Conway, S. (2004) Commensal anaerobic gut bacteria attenuate inflammation by regulating nuclear-cytoplasmic shuttling of PPAR-gamma and RelA. Nat. Immunol. 5, 104–112.
87 Neish, A. S., Gewirtz, A. T., Zeng, H., Young, A. N., Hobert, M. E., Karmali, V., Rao, A. S. and Madara, J. L. (2000) Prokaryotic regulation of epithelial responses by inhibition of IkappaB-alpha ubiquitination. Science 289, 1560–1563.
88 Oyama, J., Blais, C. Jr., Liu, X., Pu, M., Kobzik, L., Kelly, R. A. and Bourcier, T. (2004) Reduced myocardial ischemia-reperfusion injury in toll-like receptor 4-deficient mice. Circulation 109, 784–789.
89 Shishido, T., Nozaki, N., Yamaguchi, S., Shibata, Y., Nitobe, J., Miyamoto, T., Takahashi, H., Arimoto, T., Maeda, K., Yamakawa, M., Takeuchi, O., Akira, S., Takeishi, Y. and Kubota, I. (2003) Toll-like receptor-2 modulates ventricular remodeling after myocardial infarction. Circulation 108, 2905–2910.