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Pathophysiology 21 (2014) 81–93

ISP PATHOPHYSIOLOGY

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Toll-like receptor regulation of intestinal development and inflammation in the pathogenesis of necrotizing enterocolitis

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Abstract

Toll-like receptors (TLRs) are a structurally related family of molecules that respond to a wide variety of endogenous and exogenous ligands, and which serve as important components of the innate immune system. While TLRs have established roles in host defense, these molecules have also been shown to play important roles in the development of various disease states. A particularly important example of the role of TLRs in disease induction includes necrotizing enterocolitis (NEC), which is the most common gastrointestinal disease in preterm infants, and which is associated with extremely high morbidity and mortality rates. The development of NEC is thought to reflect an abnormal interaction between microorganisms and the immature intestinal epithelium, and emerging evidence has clearly placed the spotlight on an important and exciting role for TLRs, particularly TLR4, in NEC pathogenesis. In premature infants, TLR4 signaling within the small intestinal epithelium regulates apoptosis, proliferation and migration of enterocytes, affects the differentiation of goblet cells, and reduces microcirculatory perfusion, which in combination result in the development of NEC. This review will explore the signaling properties of TLRs on hematopoietic and non-hematopoietic cells, and will examine the role of TLR4 signaling in the development of NEC. In addition, the effects of dampening TLR4 signaling using synthetic and endogenous TLR4 inhibitors and active components from amniotic fluid and human milk on NEC severity will be reviewed. In so doing, we hope to present a balanced approach to the understanding of the role of TLRs in both immunity and disease pathogenesis, and to dissect the precise roles for TLR4 in both the cause and therapeutic intervention of necrotizing enterocolitis.

Keywords: Toll-like receptor 4; Necrotizing enterocolitis; Intestinal inflammation; Epithelium; Endothelium; Amniotic fluid

1. Introduction

The recognition and clearance of invading microbes depends on the coordinated activities of both the innate and adaptive immune systems. The Toll-like receptors (TLRs) play key roles in the production of innate inflammatory cytokines and also in the regulation of adaptive immune responses, and have thus emerged as important components of host defense. In addition to their signature roles in the regulation of host defense in, TLRs have also been shown to play important roles in the induction and progression of various inflammatory diseases, in part through their signaling properties on both hematopoietic as well as non-hematopoietic cells. A particularly striking example of the role of TLRs in disease pathogenesis is necrotizing enterocolitis (NEC), a severe and often fatal disease that affects preterm infants whose development requires the activation of TLR4 signaling within the intestinal epithelium. In addition to

Abbreviations: TLR, Toll-like receptor; NEC, necrotizing enterocolitis; PAMP, pathogen-associated molecular pattern; DAMP, damage-associated molecular pattern; MyD88, myeloid differentiation factor 88; TRIF, TIR domain-containing adaptor protein inducing IFN-β; NF-κB, nuclear factor-κB; IFN, interferon; LPS, lipopolysaccharide; HMGB1, high-mobility group protein B1; IBD, inflammatory bowel diseases; TOLLIP, Toll-interacting protein; SIGIRR, single immunoglobulin IL-1R-related molecule; IRAK-M, IL-1R-associated kinases M; PPARγ, peroxisome proliferator activated receptor-γ; SLPI, secretory leukocyte peptidase inhibitor; MD-2, myeloid differentiation protein-2; RhoA, Ras homolog gene family, member A; eNOS, endothelial nitric oxide synthase; HSP70, heat shock protein 70; NOD2, nucleotide-binding oligomerization domain-2; MDP, muramyl-di-peptide; SMAC-DIABLO, second mitochondria-derived activator of caspase/direct inhibitor of apoptosis-binding protein with low PI.

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0928-4680/$ – see front matter © 2013 Published by Elsevier Ireland Ltd.
http://dx.doi.org/10.1016/j.pathophys.2013.11.007

the roles of TLR4 in driving a pro-inflammatory program, recent data also implicate TLR4 activation in the regulation of intestinal differentiation. We now evaluate the signaling properties of TLR4 within both hematopoietic and non-hematopoietic cells, and summarize the role of TLR4 in the pathogenesis of NEC. We further highlight recent findings for TLR4 in the regulation of intestinal development and inflammation in its pathogenesis. We close by discussing the exciting possibility of targeting TLR4 and its related downstream signaling cascades as novel potential strategies for this devastating disease and others.

2. General introduction to the TLRs

TLRs are mammalian homologues of the Toll protein identified from Drosophila [1] which was subsequently found to exert anti-fungi functions in a landmark paper by Hoffman and colleagues [2]. TLRs represent a large family of transmembrane molecules which play a pivotal role in innate immune responses against a broad range of pathogens including bacteria, mycobacteria, mycoplasma, fungi and virus [3]. Since the first human TLR was identified in 1997 by Janeway and colleagues [4], 10 TLRs have been identified in humans and 13 in mouse [5–7]. TLRs are expressed on various hematopoietic cells, including macrophages, dendritic cells, B cells [8] and T cells [9], and to a lesser extent, on non-immune cells, including epithelial cells, endothelial cells and fibroblasts [10,11]. TLRs are present on the cell surface – as is the case for TLR1, TLR5, TLR6, TLR10 and TLR12 [3,12], or intracellularly within endosomes – as is the case for TLR3, TLR7, TLR8 and TLR9 [12], or both on the cell surface and intracellularly – as is the case for TLR2 [13], TLR4 [14–18] and TLR11 [19], or in endosomes – as is the case for TLR13 [20]. Each TLR detects distinct ligands that may be exogenous and thus pathogen related – the so called pathogen-associated molecular patterns (PAMPs), and also endogenous ligands that are released during injured states, and are termed damage-associated molecular patterns (DAMPs). Examples of PAMPs include lipoproteins (TLR1, TLR2 and TLR6), pathogen nucleic acids (TLR3, TLR7, TLR8 and TLR9), lipopolysaccharide (TLR4), and flagellin (TLR5) [3,10], while examples of DAMPs include proteins and peptides (TLR1, TLR2, TLR4, TLR7 and TLR8), fatty acids and lipoproteins (TLR2 and TLR4), proteoglycans and glycosaminoglycans (TLR2 and TLR4) and nucleic acids and protein-nucleic acids complexes (TLR3, TLR7, TLR8 and TLR9) [21]. After ligand binding, TLRs dimerize and recruit adaptor molecules in a myeloid differentiation factor 88 (MyD88)-dependent or TIR domain-containing adaptor protein inducing IFN-β (TRIF)-dependent manner [22,23]. Subsequent downstream signaling leads to the translocation of nuclear factor-κB (NF-κB) from the cytoplasm to the nucleus, and the release of inflammatory cytokines, chemokines and type I interferons (IFNs) [24–27]. In parallel, TLR activation leads to the recruitment of neutrophils [28,29] and to the activation of macrophages [30], resulting in specific innate and adaptive immunological responses. While TLRs have been well characterized on hematopoietic cells, they are also expressed on various non-hematopoietic cells, including epithelial cells, endothelial cells and fibroblasts, where they may contribute to both host defense and disease development. We will now review the important functions of TLRs in hematopoietic and non-hematopoietic cells, in order to understand the central roles that these molecules play both in host defense and in disease pathogenesis.

3. TLR signaling in hematopoietic cells

TLRs are highly expressed on hematopoietic cells, including macrophages, dendritic cells, neutrophils and lymphocytes, and TLR activation results in the initiation of both innate and adaptive responses [10,11]. TLRs activate macrophages to produce pro-inflammatory cytokines and antimicrobial proteins and peptides [31], and to trigger the autophagy pathway to clear the ingested organism [30]. Engagement of hematopoietic cell TLRs also produces type I IFNs [3,7], initiates phagocytosis of bacteria and viruses [32], and augments macrophage bactericidal activity [33]. TLRs induce dendritic cell maturation and activation, which migrate to the draining lymph nodes, presenting the antigen naïve T cells which induces regulatory T cell subsets [8,34]. In parallel, the engagement of B cell TLRs induces T-independent antibody responses [35], and TLRs are involved in the reprogramming of regulatory T cells to T helper cells, which plays a role in modulating the adaptive immune defense against certain pathogens [34].

4. TLR signaling in non-hematopoietic cells

TLRs are also expressed on a variety of non-hematopoietic cells, including epithelial cells and endothelial cells. In the kidney, TLRs (TLR1–6 and TLR11) are found on renal epithelial cells [36–39], and TLRs contribute to the pathogenesis of a number of renal diseases [40–42]. Upon lipopolysaccharide (LPS) stimulation [37], renal tubular epithelial cells secrete CC-chemokines, leading to subsequent leukocyte infiltration and tubular injury during bacterial sepsis [36], and TLR4 on intrinsic renal cells is shown to be required for the initiation of antibacterial immunity during renal infection [42]. Moreover, in renal tubular epithelial cells, bacterial infections release β-defensins [40,41], small antimicrobial peptides which are also endogenous ligands for TLR4 [43]. TLRs are also found on the renal endothelial cells, and renal vascular endothelial cells are shown to initiate early inflammatory responses in the setting of kidney injury [44]. TLR4 can regulate early endothelial activation during ischemic acute kidney injury, and the endogenous TLR4 ligand high-mobility group protein B1 (HMGB1) released by injured renal cells up-regulates the expression of