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Mediators of Tyrosine Phosphorylation in Innate Immunity: From Host Defense to Inflammation onto Oncogenesis

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Abstract

Cells respond to extracellular cues through a variety of receptors on the surface. These signals once transduced across the cell membrane, activate protein tyrosine kinases, which through phosphorylation of substrates on key tyrosine residues, are able to control cellular growth, activation and differentiation pathways. Recent data suggest that protein tyrosine kinases are critical in integrating signals from various cellular receptors, including pathogen detection receptors that mediate the host innate immune response. In this article, we have reviewed the roles of tyrosine kinases of the Tec, FAK, Fps, Fer, Syk, Src and TAM-receptor families in toll-like receptor signaling. The shared roles of these tyrosine phosphorylation mediators in host defense, inflammation, autoimmune disease and oncogenesis provides promising avenues for the use of their inhibitors in multiple disorders.

Keywords

Toll-like receptors; tyrosine kinases; innate immunity; phosphorylation; cytokine release

Introduction

Pathogen detection by toll-like receptors (TLRs) is crucial for the initiation and regulation of innate immune responses. Engagement of these receptors signals the activation of transcription factors, such as NFκB, *via* activation of canonical IκB kinases (IKKs) and IKK-related kinases (ex. TANK- binding kinase 1, TBK1 and IKK*i*) (reviewed in [1,2]). This leads to the induction of molecules, such as TNFα, and IL6, that are required for pathogen clearance. Significantly, these molecules are also implicated in cell survival, proliferation and angiogenesis signaling pathways. Inflammatory events can promote tumorigenesis. NFκB activation is a hallmark of most cancers.

Protein tyrosine kinases (PTKs) are compelling targets in human cancer. These enzymes regulate multiple cellular processes that contribute to tumor development and progression; such as cell growth, differentiation, migration and apoptosis. Roles for multiple PTKs in early innate immune signaling have recently emerged. This article reviews our current knowledge of regulation of TLR signaling by PTKs. Many of these are targets for therapeutic intervention of immune disorders.

A role for PTKs in early innate immune response was first suggested by the observation that they are required for the induction of pro-inflammatory cytokines IL-1β, IL-6 and TNFα in

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response to endotoxin in murine macrophages [3]. Conflictingly, inhibition of LPS-mediated tyrosine kinase activity with broad spectrum PTK inhibitor, genistein, failed to inhibit LPS-mediated NFκB translocation [4]. In the past three years, roles for multiple PTKs have emerged in TLR signaling (summarized in Table 1).

Tec Tyrosine Kinases

A role for bruton's tyrosine kinase (Btk), the prototypical member of the tec family of tyrosine kinases, in LPS signaling was first suggested by studies from xid mice that lack a functional Btk [5]. Macrophages from xid mice show poor nitric oxide induction and reduced production of inflammatory cytokines IL1β and TNFα in response to LPS challenge. LPS induces tyrosine phosphorylation of Btk, presumably *via* Src family kinases [6], and activates its tyrosine kinase activity. Btk interacts with TIR domains of many TLRs including TLR4 [7], and adaptor proteins MyD88 and Mal [8,9]. Upon TLR2 and TLR4 stimulation, Mal is phosphorylated by Btk [9] and then interacts with SOCS-1, which results in Mal polyubiquitination and subsequent degradation [10]. Deficient Btk function selectively impairs dendritic cell cytokine induction in response to viral single-stranded RNAs that activate TLR8 [11].

A second tec family member, Bmx, is required for TLR4 induced IL6 production in macrophages and rheumatoid arthritis synovial cells, *via* mRNA stabilization [12,13]. A third tec family kinase member, Etk, is present in epithelial and endothelial cells and fibroblasts. Etk activation is regulated by focal adhesion kinase (FAK) through interaction between the PH domain of Etk and the FERM domain of FAK [14]. Etk is implicated in crosstalk between integrin $\alpha_5\beta_1$/FAK and MyD88 pathways in fibroblast-like synoviocytes and plays a role in IL-6 synthesis [15]. In coimmunoprecipitation experiments, Etk is associated with MyD88, FAK and Mal, and Etk phosphorylation is dependent on FAK kinase activity.

Focal Adhesion Kinases

FAK can trigger inflammatory responses [16]. Protein I/II, a cell wall component from oral streptococci, upon binding to integrin $\alpha_5\beta_1$, induces inflammatory mediators such as IL-6 and IL-8 in human monocytes, epithelial cells, endothelial cells and fibroblasts [16,17], and the signaling leading to cytokine release involves FAK. FAK is also involved in invasin-mediated bacterial uptake [18]. There is considerable cross-talk between integrin/FAK and TLR pathways; LPS induces FAK phosphorylation in a murine monocytic cell line [19]. TLR2 ligand Pam_3CSK_4 also induces FAK phosphorylation at Tyr397, and requires FAK for cytokine release [20]. TLR2 and TLR4 agonists also induce tyrosine phosphorylation of the proline-rich tyrosine kinase 2 (Pyk2), which in turn increases tyrosine phosphorylation of the adaptor protein paxillin. Paxillin is involved in binding to Pyk2, vinculin and FAK through MyD88-dependent and independent pathways [21]. MyD88 is however not essential for FAK autophosphorylation, as it is phosphorylated in $MyD88^{-/-}$ macrophages that were activated with LPS or proteinI/II. MyD88 and FAK, but not TLRs 2, 4 or 6 are involved in cytokine release mediated by proteinI/II. Furthermore, LPS-induced cytokine release also depends on presence of both FAK and MyD88, suggesting that FAK may play a general role in proinflammatory cytokine release [20].

Pyk2 acts as a molecular switch to overcome suppression of leukocyte oxidant generation by cell adhesion [22]. Prior exposures of neutrophils to cytokines and inflammatory mediators (e.g. TNF-α, GM-CSF), overcomes the adhesion-mediated suppression of reactive oxygen species formation. LPS can induce human T cells to adhere to fibronectin *via* TLR4 signaling, and the human T cell response to LPS depends on protein kinase C and involves the phosphorylation of Pyk2 within 10 min [23] of challenge.

Fps/Fes and Fer

Knockout mouse models have provided evidence for the involvement of tyrosine kinase Fps [24] and Fer [25] in the innate immune response. Fps/Fes knockout mice are hypersensitive to systemic LPS challenge. Fer-deficient mice display increased leukocyte recruitment at sites of localized LPS challenge, and enhanced intestinal barrier dysfunction in response to LPS [26]. Fps null mice display increased mortality in response to intraperitoneal challenge with LPS. There is an increase in circulating TNFα levels in LPS-challenged Fps-null mice, which show an enhanced NFκB signaling response. These mice have a defective down-regulation of TLR4, which correlates with a general role for Fps in internalization [27]. A role for Fps has also been determined in leukocyte recruitment to areas of inflammation [28]; and fps null mice display increased neutrophil rolling, adhesion and extravasation subsequent to LPS challenge.

Spleen Tyrosine Kinase (Syk)

Tyrosine kinase Syk plays a role in TLR4 dependent macropinocytosis and lipid accumulation in macrophages [29]. Minimally modified LDL induces Syk association with TLR4, as well as Syk phosphorylation. Syk-deficient dendritic cells (DCs) show increased inflammatory cytokine production, maturation and antigen presentation [30]. Immunoreceptor tyrosine-based activation motif (ITAM) containing adaptors, DAP12 and FcεRIγ-chain (FcRγ), are both required for negative regulation of TLR responses in bone marrow derived DC's [30], and DAP12 negatively regulates TLR signaling in macrophages [31,32] and plasmacytoid DC's [33].

Syk is implicated in direct modulation of multiple TLR responses. Syk associates with TLRs 4 [34] and 9 [35]. Stimulation of TLR9 with CpG, for example, induces IL12p70 production, that is dependent on Syk [36]. Similarly, stimulation of TLR4 with LPS induces release of IL-10 and IL12-p70, which is dependent on Syk [34]. More significantly, emerging data implicates a role for Syk in collaborative TLR signaling. The fungal β-glucan receptor, Dectin-1, a C-type lectin, binds to yeast and signals through the kinase Syk and the adaptor CARD9 to induce production of IL-10 and IL-2 in DCs [36]. The interaction between Syk and Dectin-1 occurs through a novel mechanism [37] and $Syk^{-/-}$ DCs do not make IL-10 or IL-2 upon yeast stimulation but produce IL-12, indicating that the Dectin-1/Syk and Dectin-1/TLR2 pathways can operate independently. Dectin-1 induced Syk activation is essential for the production of pro-inflammatory cytokines and the release of reactive oxygen by *Mycobacterium abscessus* infected macrophages. *M. abscessus* activation also requires a physical interaction between TLR2 and dectin-1 [38]. Collaborative cytokine production (TNFα, MIP-1α and MIP-2) induced through dectin-1 and TLRs 2, 4, 5, 7 and 9 [39] requires Syk. Deficiency of either Syk or MyD88 abolishes collaborative responses. In neutrophils, an adhesion receptor (CEACAM3), acts to capture and engulf invasive bacteria. Bacterial binding to CEACAM3 also causes recruitment of Syk, and its phosphorylation [40].

Src Family Kinases (SFK)

A role for PTKs in LPS signaling was first established when it was reported that myeloid cells stimulated with LPS showed enhanced expression of Src kinases Hck and Lyn [41,42]. Src kinases were more directly implicated in LPS signaling to TNFα and *i*NOS in murine macrophages, based on effects seen with Src inhibitor PP1 [43]. Endotoxin tolerant cells show suppressed LPS-mediated TLR4 tyrosine phosphorylation, and recruitment of Lyn kinase to TLR4. However, their importance in LPS signaling in macrophages has been questioned by the studies from $Hck^{-/-}Fgr^{-/-}Lyn^{-/-}$ mice, which show no major impairment in LPS-mediated activation [44].

A systemic inhibition of Src kinases can attenuate LPS-induced lung injury, suggesting a role for SFK's in acute lung injury [45]. Src inhibitors, PP2, SU6656 and tyrphostin A1, block LPS-dependent cytokine and chemokine production in the lung and in serum. In human lung microvascular endothelial cells, LPS recognition by TLR4 activates *c*-Src, Fyn and Yes, which contribute to tyrosine phosphorylation of zonula adherens proteins and enhance endothelial paracellular permeability [46]. These three SFKs play redundant but not totally compensatory roles in LPS-induced barrier dysfunction. *c*-Src is also activated by dsRNA in human monocyte derived dendritic cells, and is recruited to TLR3 in a dsRNA dependent manner [47]. Src kinase-deficient cells show no activation of dsRNA induced IRF3 and STAT1, suggesting a critical role in antiviral immunity. Additionally, PP2 inhibits TNF production upon engagement of TLRs 1, 2, 4, 5 and 6; and marginally to TLR7/8 indicating SFKs may be common regulators of many TLR signaling in primary human monocyte derived macrophages [48].

SFKs play a key role in autoimmunity. Lyn-deficient mice develop systemic lupus erythematosus (SLE) [49,50]. Additionally, lower levels of Lyn have been discovered in B cells of human SLE patients [51]. A recent correlation suggests that autoimmune disease in Lyn-deficient mice is MyD88-dependent [52]. TLR signaling plays an essential role in the development of pathogenic autoantibodies and kidney disease in $Lyn^{-/-}$ mice. The autoimmune phenotyes are completely eliminated in $MyD88^{-/-}$ $Lyn^{-/-}$ mice. A role for TLR signaling in the development of autoimmune diseases is still in its early stages. Notably, a single nucleotide polymorphism in TLR5 has been associated with SLE resistance [53].

C-terminal src kinase (Csk), a negative regulator of Src kinases, also plays a role in LPS response in mouse macrophages. Csk knockdown cells show reduced protein expression levels of tyrosine kinase Fgr, and reduced secretion of cytokines IL6 and TNFα [54].

Tyro, Axl and Mer (TAM) Receptor Tyrosine Kinases

Mer receptor tyrosine kinase is involved in apoptotic cell clearance, by mediating an engulfment pathway in concert with $\alpha_v\beta_5$ integrin. It also acts as a negative regulator for inflammation by down-modulating pro-inflammatory signals (IL1β, TNFα and IL-12) mediated from LPS-TLR4 signaling [55,56]. Significantly, the trans-inhibition of NFκB activation by Mer occurs independent of cytosolic IκB phosphorylation and p65/RelA sequesteration [57]. Mice that express mutant Mer (Mer^{KD}), when backcrossed into the C57/bl/6 background develop an age-dependent autoimmune disease characterized by the loss of ability to ingest apoptotic cells [58].

Loss of function of three TAM receptors, Tyro3, Axl and Mer, is associated with dysregulation of the immune response [59]. Additionally, $Tyro3^{-/-}Axl^{-/-}Mer^{-/-}$ triple mutant mice display broad spectrum autoimmune disease. $Mer^{-/-}$ single mutants are hypersensitive to LPS-induced endotoxic shock [56]. Tyro3, Axl and Mer broadly inhibit both TLR and TLR-induced cytokine receptor cascades [60]. TAM inhibition of inflammation is transduced through the type I interferon (IFN) receptor and its associated transcription factor STAT1, and this pathway controls the phased attenuation of antigen presenting cell activation.

Tyrosine Phosphatases

SHP-1 (A002156) and SHP-2 (A002157) are intracellular protein tyrosine phosphatases that contain two tandemly linked Src homology 2 domains at their amino termini, followed by a catalytic domain. Recently, SHP-1 was shown to differentially regulate the production of proinflammatory cytokines and type I IFN [61]. SHP-1 inhibits TLR-mediated production of cytokines by suppressing the activation of MAPs and NFκB, but it increased TLR and

RIG-1 activated type I IFN by directly binding to the kinase domain of IRAK1 and inhibiting its activity. Significantly, the phosphatase activity of SHP-1 is not required for inhibition of IRAK1 activation. This study suggests role for SHP1 in immune homeostasis. Its relative SHP2 more narrowly regulates TLR3 induced inflammatory cytokines and IFNβ, without affecting TLR2 or 9 signaling. SHP2 targets TBK-1, the kinase that phosphorylates IRF3 [62].

Tyrosine Kinase Inhibitors in Autoimmune Disease

Drugs targeting kinases e.g. Lck and Syk are currently undergoing clinical trials for the treatment of diseases related to inflammation and autoimmunity (reviewed in [63]). In human peripheral blood macrophages and monocytes, imatinib, a small molecule PTK inhibitor, inhibits LPS –induced production of TNFα through a *c*-Fms independent mechanism. TNFα is a driver of autoimmune tissue injury in a spectrum of autoimmune diseases including rheumatoid arthritis, Crohn disease, psoriasis and multiple sclerosis [64]. Imatinib can inhibit a narrow spectrum of PTKs (Abl, *c*-Fms, *c*-Kit, PDGFR αβ and Lck) at submicromolar concentrations [65]. This ability of Imatinib and other PTK inhibitors to inhibit multiple kinases, could actually be beneficial for treatment of autoimmune diseases, where multiple unmutated kinases (unlike mutations associated with malignancy) play a role.

Summary

Emerging data indicate that PTKs integrate signals from various receptors to TLRs, thereby defining critical regulatory mechanisms based on recognition of multiple ligands in tandem (see Fig. (1)). The Tec family kinases have been studied in some detail, and not only play direct roles in transduction of TLR signals *via* interactions with TIR domains; but like the FAK family, interface signals from adhesion receptors such as the integrins to TLRs. Tyrosine kinase Syk integrates collaborative signals between fungal receptors and multiple TLRs. SFKs play a role in tolerance, membrane permeability, antiviral immunity, and autoimmunity. TAM receptor tyrosine kinases feed in regulatory signals from adjacent apoptotic cells, playing a critical role in control of autoimmune disorders. In summary, tyrosine mediators of innate immunity are key regulators of an uncontrolled immune response as they interface signals from multiple cellular receptors.

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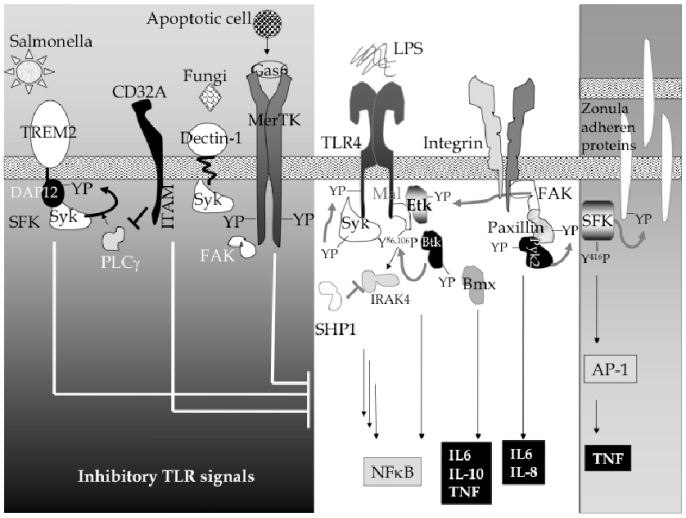

Fig. (1).

Tyrosine phosphorylation plays a role in regulation of the innate immune response through TLRs. Signals from TLR-mediated PTK activation couple with signals from recognition of other stimuli, such as the detection of fungi, apoptotic cells (grey box on the left) to determine the net host response. YP represents tyrosine phosphorylation.

Table 1

Tyrosine Kinases Implicated in TLR Signaling. Tyrosine Kinases, their Substrates, Binding Partners, Relevant Cell Types and Activities in TLR Signaling

Kinase	Substrate(s)	Binding Partners	Reported Expression/Activity in Cell Type	Regulation/Function	TLR Signaling	Reference
Btk	Mal	TLR4, MyD88, Mal	Mouse macrophages, myeloid cells	NO, IL1β, TNFα	TLR2, TLR4	[5-11]
Bmx			Macrophages and rheumatoid arthritis synovial cells	IL6	TLR4	[12,13]
Etk		FAK, MyD88, Mal	Epithelial, endothelial cells and fibroblasts	IL6	TLR4	[14,15]
FAK	Etk	Etk,	Human monocytes, epithelial cells, endothelial cells, fibroblasts	IL6, IL8	TLR2, TLR4	[16-20]
Pyk2	Paxillin	Paxillin, vinculin, FAK	Human T cells		TLR2, TLR4	[21,23]
Fps				TLR4 internalization, leucocyte recruitment. neutrophil rolling, adhesion and extravasation, TNFα	TLR4	[24,27,28]
Syk	TLR4	TLR4, 9 Dectin-1	Macrophages, dendritic cells	macropinocytosis and lipid accumulation, IL12p70 IL10 IL2	TLRs 2, 4, 5, 7, 9, Dectin-1	[29,30,34-37]
Lyn		TLR4	B cells	IL6, IL12 SLE	TLR4	[41,42,44] [51]
c-Src, Fyn, Yes	Zonal adherens proteins			endothelial paracellular permeability	TLR4	[46]
c-Src		TLR3	Dendritic cells	IRF-3 and STAT-1	TLR3	[47]
Csk			Mouse macrophages	IL6, TNFα	TLR4	[54]
Tyro3, Axl and Mer				Broad spectrum autoimmune disease. Cytokine release Type I IFNR	TLR4	[55,56,58-60]
SHP-1		Kinase domain of IRAK-1		Inflammatory cytokines and type I IFN		[61]
SHP-2		TBK-1		Inflammatory cytokines and IFNβ	TLR3	[62]