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Publication details:
Biochemical Society Transactions
v. 40
Chapter No. 5
pp. 1134-1139
0300-5127 (ISSN)

Publication Date:
2012

Publisher DOI:
http://dx.doi.org/10.1042/BST20120119

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An emerging role for LRRK2 in the immune system

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Abstract
Missense mutations in leucine-rich repeat kinase 2 (LRRK2) contribute significantly to autosomal dominant Parkinson's disease (PD). Genome-wide association studies have further suggested that mutations in LRRK2 comprise a risk factor for sporadic PD. How LRRK2 contributes to PD however, is largely unknown. Recent work has shown that LRRK2 is highly expressed in tissue and circulating immune cells and is suggestive of a potential role for LRRK2 in innate immunity. These studies and their potential implications for PD will be discussed.

Introduction

Advances in genomic sequencing have led to the discovery of a number of genes in which mutations can predispose to familial Parkinson's disease (PD) in either a recessive (PARKIN, PINK1, DJ1) or autosomal dominant (SNCA, LRRK2, VPS35) manner. Of these genes, pathogenic mutations in leucine-rich repeat kinase 2 (LRRK2) are the most prevalent genetic cause of PD [1]. Clinical analysis has established that familial PD caused by LRRK2 mutations is largely indistinguishable from sporadic PD in terms of age of onset and disease progression [2]. The most common pathogenic mutation in LRRK2 results in the substitution of Gly 2019 to Ser. Gly 2019 lies in the invariant DFG motif located before the activation loop in the protein kinase domain of LRRK2. The discovery that the G2019S mutation results in a constitutive 2-3 fold increase in the protein kinase activity of LRRK2 [3, 4] has made the development of small molecule kinase inhibitors of LRRK2 a priority. Additionally, genome wide association studies have uncovered LRRK2 mutations as risk factors for the more common sporadic form of PD [5, 6]. It is therefore plausible that LRRK2 dysfunction might occur in both familial and the more common sporadic form of PD. Despite intensive research over a number of years, the physiological function of LRRK2 has remained largely elusive. Identifying the role of LRRK2 and how mutations in this enzyme pre-dispose to PD remain a major hurdle in the progression of LRRK2 kinase inhibitors to therapy. Evidence is now accumulating that LRRK2 may play a role in immunity, in particular the inflammatory response mediated via the innate immune system. This makes an intriguing development as neuroinflammation has long been associated with the pathogenesis of PD.

The receptor interacting protein (RIP) kinase family

LRRK2, and the highly related LRRK1, are both members of the receptor interacting protein kinase (RIPK) branch of the human kinome [7] (figure 1). This family consists of seven members with LRRK1 and LRRK2 being designated RIPK6 and RIPK7 respectively [8, 9]. RIPK members 1 through 5 have been investigated to varying extents and are known to play roles in immune signalling pathways [8, 9] (figure 1). A well studied member, RIPK2, is required for the signal transduction response to invasion by intracellular bacteria. In this signalling pathway peptidoglycan moeties found on bacterial cell walls are first "sensed" by the nuclear oligomerisation domain (NOD) proteins 1 and 2 by binding to the leucine rich repeats in these proteins [10]. NOD proteins then recruit RIPK2, which mediates downstream signalling through nuclear factor kappa B (NFkB) resulting in a subsequent inflammatory cytokine response [11, 12]. Mutations in NOD2 are associated with increased susceptibility to Crohn's disease [13, 14] an inflammatory disorder of the bowel. Intriguingly, a meta analysis of Crohn's disease genome-wide association studies found that mutations in the non-coding region of the LRRK2 gene comprise a

risk factor for this disease [15]. Indeed, LRRK2 knockout mice are more susceptible to the widely used dextran sulphate sodium model of Crohn's disease [16]. Further parallels between traditional RIPK members and LRRK2 exist. In regard to RIPK2, kinase activity is required for protein stability [17, 18] a phenomenon recently described for LRRK2 [19]. For RIPK1 and RIPK3, kinase activity is required for their interaction which further mediates downstream signalling [20]. RIPK1 has also been suggested as an interacting protein for LRRK2, along with other proteins of the FADD/RIPK1 signal transduction pathway [21] (figure 2). It is therefore plausible that LRRK2, like other RIPK members, may play a role in immune signalling and/or the inflammatory response to pathogens

The expression of LRRK2 in immune cells

Results obtained by a number of investigators have demonstrated that the expression of LRRK2 varies substantially in different cell or tissue populations. In mouse tissue, LRRK2 is readily detected at the protein level in kidney and spleen with a lower expression in brain [22, 23]. In spleen tissue, LRRK2 was readily detectable in the CD19+ B-lymphocytes but not T-lymphocytes [22]. LRRK2 is also readily detectable in human EBV immortalized lymphoblasts [24] which has made these cells useful for measuring endogenous LRRK2 kinase activity and assessing the effectiveness of LRRK2 kinase inhibitors in human cells [25, 26]. An initial analysis of LRRK2 expression in primary human peripheral blood mononuclear cells (PBMCs), performed by Gardet et al., demonstrated that LRRK2 expression was highest in B-lymphocytes, followed by monocytes and then dendritic cells [27]. Additional reports have subsequently confirmed that LRRK2 is robustly expressed in human CD19+ B lymphocytes and CD14+ monocytes [28, 29]. Much lower levels of LRRK2 protein have been reported for CD3+ T-lymphocytes [27, 28]. In regard to monocytes, the highest expression of LRRK2 was seen in the CD14+/CD16+ population, which resembles a more mature or activated monocyte population [28]. Circulating monocytes can also differentiate to tissue macrophages and LRRK2 is readily detectable in the immortalized mouse RAW264.7 macrophage cell line as well as in primary mouse bone marrow derived macrophages (Dzamko). LRRK2 has also been detected at the protein level in microglia, the resident macrophages of brain, from humans [30], adult mice [31, 32] and in primary mouse microglial cultures derived from postnatal pups [31].

The regulation of LRRK2 expression in immune cells

A robust increase in the mRNA and protein expression of LRRK2 occurs when human PBMCs are treated with interferon gamma (IFNγ) [27, 28] (figure 2). In particular, IFNγ induced the expression of LRRK2 in CD19+ B-lymphocytes, CD11b+ monocytes and CD3+ T-lymphocytes [27]. IFNγ is predominantly produced by T-lymphocytes and NK cells to stimulate the activation of monocytes and consequently, treatment of isolated human monocytes with IFNγ shifted the monocyte population from CD14+CD16- to CD14+CD16+, suggesting that LRRK2 expression is upregulated during the activation of monocytes [28]. Intriguingly it has recently been reported that LRRK2 expression is also upregulated in microglia that have been activated by bacterial lipopolysaccharide (LPS) [31]. In contrast to IFNγ, that signals through the JAK-STAT pathway, LPS is an agonist for toll-like receptor (TLR) signaling, in particular TLR4. LPS induced the upregulation of LRRK2 protein in cultured microglia and positive staining for LRRK2 could be detected in activated microglia following injection of LPS into the substantia nigra pars compacta of mice

[31]. In these experiments, the increase in protein content of LRRK2 following LPS stimulation was independent of changes in the mRNA expression of LRRK2. To date however, a number of studies investigating the induction of LRRK2 mRNA and protein by LPS have reported a number of contrasting conclusions. In human PBMCs, LPS treatment resulted in a decrease in LRRK2 protein with no change in mRNA [28]. In primary bone marrow derived macrophages LPS has been reported to induce either a marked increase in LRRK2 mRNA with a very modest increase in LRRK2 protein [29], no significant change in LRRK2 protein (Dzamko) or the complete disappearance of LRRK2 protein within 1h of LPS treatment [16]. Reasons for such conflicting results could include inconsistency between LRRK2 antibodies used, concentration of LPS and time points investigated. A potential further complication is that the mRNA and protein expression of LRRK2 has also been reported to increase following treatment of PBMCs with IFNβ and TNFα [28], both of which are secreted by microglia / macrophages following treatment with LPS.

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LRRK2 is phosphorylated during toll-like receptor signaling
Further evidence that LRRK2 is involved in immune signaling comes from the discovery that LRRK2 is directly phosphorylated following activation of Toll-like receptors (TLR's) (Dzamko) (figure 2). Like the NOD proteins described above, TLR's also "sense" particular pathogen associated molecular patterns via their leucine-rich repeat domains [33-35]. Activation of TLR signaling mediates the inflammatory response to foreign pathogens through increased expression of NFkB mediated cytokines such as TNFα and IL-6 [36]. LRRK2 is phosphorylated on serines 910 and 935 within 30 minutes of activation of TLR's that signal through the MyD88 adaptor protein. This equates to all TLR's except TLR3, which senses double stranded viral RNA and signals through the TRIF adaptor protein [37]. The kinases responsible for LRRK2 phosphorylation are the IKK members. The non-canonical IKK members, TBK1 and IKKε, show a marked preference for phosphorylating LRRK2 over the canonical IKK members IKKα and IKKβ. Recent work has shown that inhibition of TBK1/IKKε results in loss of a negative feedback loop responsible for suppressing the activation of IKKα and IKKβ [38]. Inhibition of TBK1/IKKε therefore increases the catalytic activity of IKKα and IKKβ and this is likely the reason that inhibition of all four IKK members is required to block the phosphorylation of LRRK2 on Ser910 and Ser935 following TLR activation. To date, the physiological relevance of this phosphorylation event remains unknown. It has previously been demonstrated that phosphorylation on serines 910 and 935 of LRRK2 regulates the interaction of LRRK2 with 14-3-3 proteins [26]. Furthermore modulation of Ser910 and Ser935 influences the subcellular localization of LRRK2, at least in an overexpression system [25, 26, 39]. Interestingly, a portion of LRRK2 has been reported to localize to *S. typhmurium* during bacterial infection of RAW cells [27] suggesting the localization of LRRK2 may be important for its function.

LRRK2 regulation of TLR agonist-induced inflammatory cytokine secretion
The presence of LRRK2 in macrophages and microglia has prompted investigators to probe for any potential role of LRRK2 in the regulation of the inflammatory immune response. Over expression of LRRK2 can reportedly activate the transcription of NFκB in luciferase reporter assays in a manner independent of LRRK2 kinase activity [27]. The transcription factor NFκB regulates the expression of a number of pro-inflammatory cytokines including TNFα, IL-6 and IL-12. Support for a role of LRRK2 in the regulation of NFκB transcription comes from two recent studies using

microglia. Firstly, overexpression of human LRRK2 harboring the R1441G mutation in mice resulted in a significant increase in the production of TNFα by microglia compared to wild type mice [32]. Secondly, lentiviral knockdown of LRRK2 in microglia reduced the production of TNFα in response to LPS [31]. Interestingly, in this study, inhibition of LRRK2 kinase activity with two structurally distinct small molecule inhibitors also reduced the secretion of TNFα in response to LPS [31]. Surprisingly however, the LRRK2 knockdown studies in microglia are at odds with a number of studies utilizing BMDMs from wild type and LRRK2 deficient mice. The first study in this area found no difference in the production of IL-6 between wild type and LRRK2 deficient macrophages treated with LPS [29]. Subsequent independent studies have also found no difference in cytokine production between wild type and LRRK2 deficient BMDMs treated with a number of toll-like receptor agonists, including LPS [16] (Dzamko). Although BMDMs share many phenotypic features of microglia, it remains a possibility that LRRK2 may play a more significant role in the regulation of inflammatory cytokine production in microglia as opposed to macrophages. It is thought for example that inflammatory cytokine secretion is more tightly regulated in microglia, as neurons are particularly vulnerable to inflammatory insult.

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LRRK2 as a regulator of NFAT

In addition to a potential role in TLR-mediated immune signaling, LRRK2 has recently been proposed to negatively regulate the transcription factor NFAT in a TLR-independent manner [16] (figure 2). Treatment of LRRK2 deficient bone marrow derived macrophages with the TLR2/dectin-1 agonist zymosan, but not the TLR2 only agonist Pam_3CSK_4, resulted in increased secretion of IL-12 and IL-6. Increased cytokine secretion was associated with increased nuclear localization of NFAT that was not dependent on NFκB or MAPK signaling [16]. In contrast, the phosphorylation of LRRK2 at Ser910 and Ser935 following zymosan treatment is dependent on the IKK family kinases, whilst the dectin-1 specific agonist, curdlan, cannot induce the phosphorylation of LRRK2 (Dzamko). These results suggest that LRRK2 may modulate cytokine secretion to different agonists of at least two independent innate immune signaling pathways.

Inflammation and Parkinson's disease

The identification of LRRK2 as a component of immune signalling is potentially important as numerous studies have identified changes in lymphocyte, monocyte, natural killer cell and pro-inflammatory cytokine levels in blood, cerebrospinal fluid and CNS tissue from patients with PD (for more detailed reviews see [40, 41]). Of particular interest is microglial mediated neuroinflammation, which is thought to underpin the ongoing cell death over time in patients with PD [42]. Conditioned media from microglial cultures activated by TLR agonists is toxic to neuronal cells. Furthermore, both central and peripheral LPS administration can induce neuronal loss and PD symptoms in animal models [43, 44]. Of further importance is that LRRK2 is not the first PD susceptibility gene to be associated with inflammation. In particular, recent work has suggested that alpha-synuclein can induce the secretion of inflammatory cytokines [45] and even increase the expression of TLR's [46]. Again peripheral LPS exacerbated the inflammatory effect of central alpha-synuclein [47] indicating a complex interplay between the peripheral and central immune systems.

Conclusions

Although an area in its infancy, increasing evidence suggests that LRRK2 may comprise a novel component of innate immune / inflammatory pathways (figure 2). More work is required to define this role and to resolve discrepancies observed in regard to the upregulation of LRRK2 protein by LPS. Further work will also be required to determine why differences in cytokine secretion between LRRK2 deficient macrophages and microglia are observed, and ultimately which is more important in regard to the pathological progression of PD. It is also possible that LRRK2 has a function beyond the regulation of inflammatory cytokine secretion. TBK1 has recently been shown to phosphorylate optinuerin following activation of TLR's and this event is required for autophagy [48]. A number of reports have also implicated LRRK2 in the regulation of autophagy [49, 50]. LRRK2 has also been reported to localize to microtubules [51] and may play a role in cytoskeletal reorganization during macrophage/microglia migration and/or phagocytosis. Furthermore, the function of LRRK2 in other immune cells, in particular, B-lymphocytes, has yet to be explored. Future studies investigating how pathogenic Parkinson's disease mutations and LRRK2 kinase inhibitors impact on peripheral and central immunity seem warranted.

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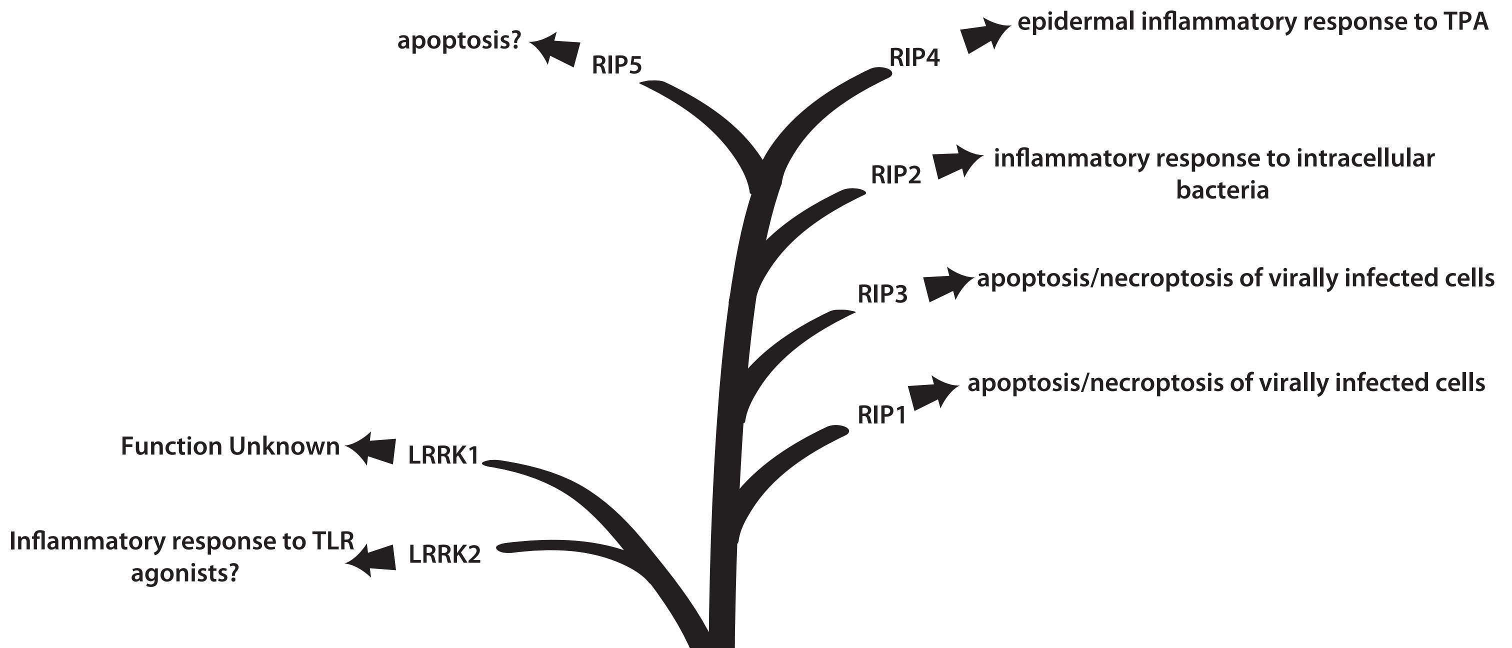
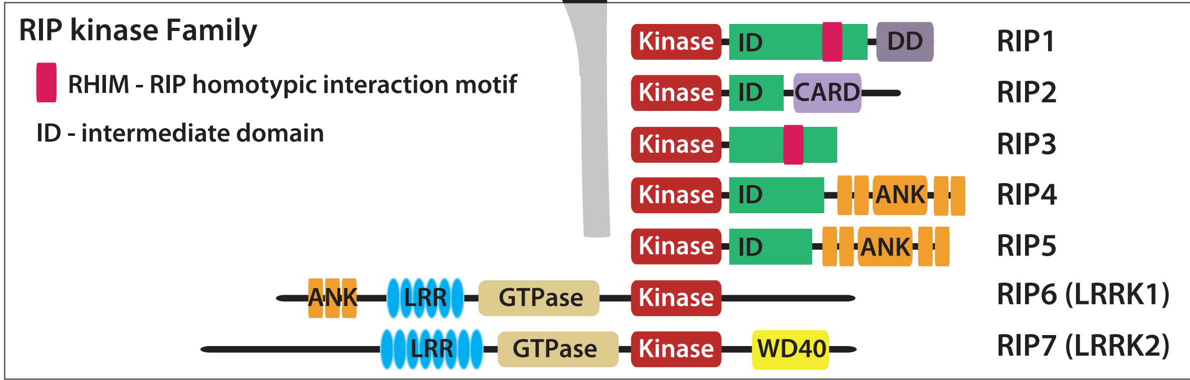
Figure legends

Figure 1. LRRK2 is a member of the Receptor Interacting Protein (RIP) Kinase family. The RIP kinase branch of the tyrosine kinase like (TKL) family of the human kinome demonstrates the relationship of LRRK1 and LRRK2 to other RIP kinase family members. RIP kinases are known to play a number of roles in the immune system including the regulation of programmed cell death, mediated by a RIP1-RIP3 complex and the inflammatory response to intracellular bacteria, mediated by RIP2. The domain structure of all RIP kinase family members is also shown.

Figure 2. Proposed roles for LRRK2 in immune signaling. A number of roles have been proposed for LRRK2 in immune signaling. LRRK2 can act as a negative regulator of NFAT by sequestering NFAT into the cytoplasm. The LRRK2-NFAT complex is disrupted following activation of Dectin receptors allowing NFAT to enter the nucleus and promote the expression of IL-6 and IL-12. LRRK2 mRNA and protein levels are upregulated following activation of the IFNγ receptor and downstream JAK-STAT signaling. LRRK2 is found in complex with FADD, RIP1 and caspase 8. When the death receptor FAS is activated, caspase 8 is released from the complex to initiate apoptosis. LRRK2 is phosphorylated following activation of TLR receptors that signal through MYD88 and may modulate inflammatory cytokine expression in microglia.

Acknowledgements
We thank Heidi Cartwright for assistance with figure preparation. Nicolas Dzamko is supported by a NHMRC postdoctoral training fellowship. Glenda Halliday is a NHMRC supported senior principal research fellow.


apoptosis?
RIP5
RIP4
epidermal inflammatory response to TPA
RIP2
inflammatory response to intracellular bacteria
RIP3
apoptosis/necroptosis of virally infected cells
RIP1
apoptosis/necroptosis of virally infected cells
Function Unknown
LRRK1
Inflammatory response to TLR agonists?
LRRK2


RIP kinase Family
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ID - intermediate domain
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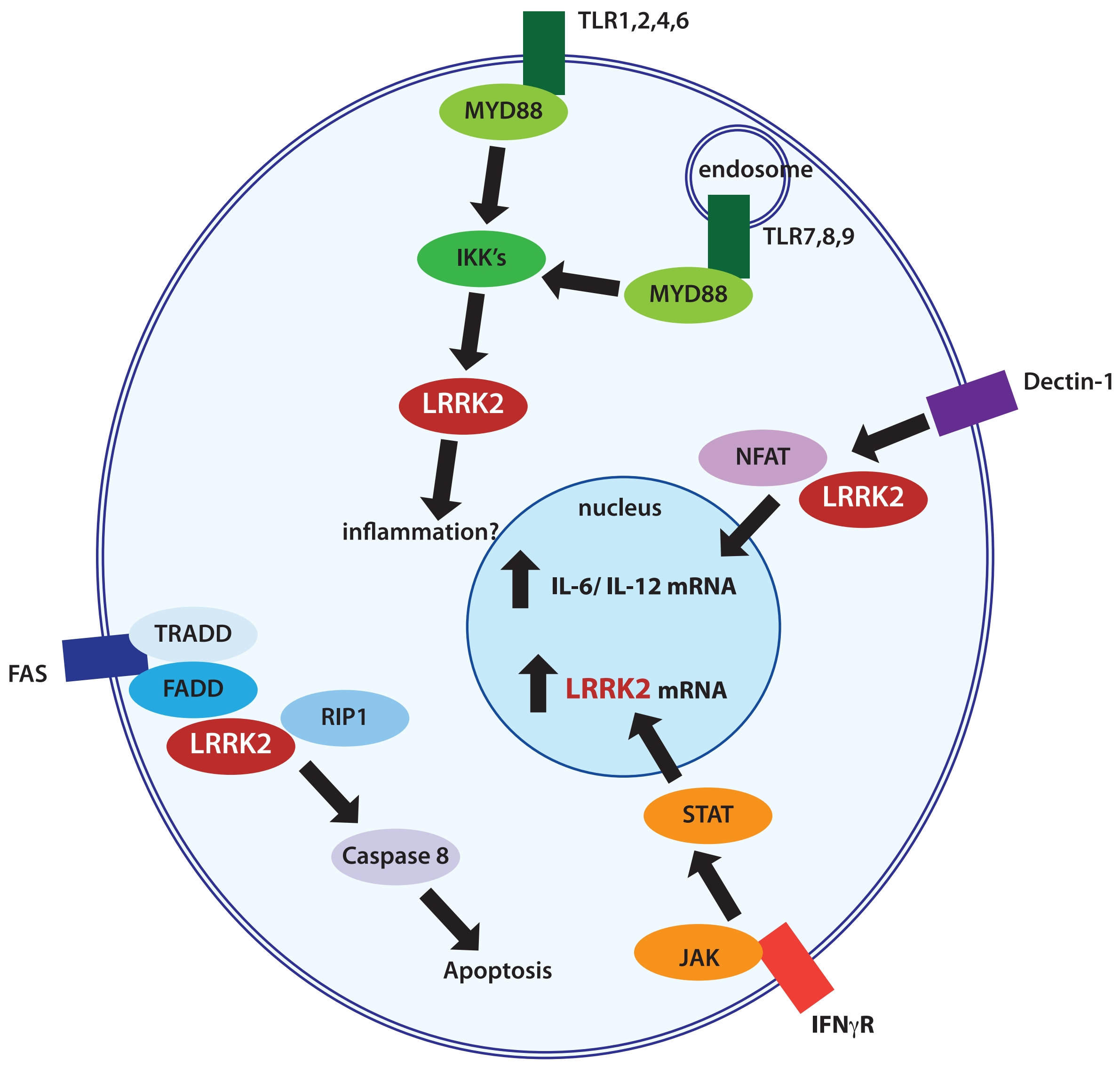
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