Modulation of Tumor Necrosis Factor and Interleukin-1-dependent NF-κB Activity by mPLK/IRAK*

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The innate immune response is an important defense against pathogenic agents. A component of this response is the NF-κB-dependent activation of genes encoding inflammatory cytokines such as interleukin-8 (IL-8) and cell adhesion molecules like E-selectin. Members of the serine/threonine innate immune kinase family of proteins have been proposed to mediate the innate immune response. One serine/threonine innate immune kinase family member, the mouse Pelle-like kinase/human interleukin-1 receptor-associated kinase (mPLK/IRAK), has been proposed to play an obligate role in promoting IL-1-mediated inflammation. However, it is currently unknown whether mPLK/IRAK catalytic activity is required for IL-1-dependent NF-κB activation. The present study demonstrates that mPLK/IRAK catalytic activity is not required for IL-1-mediated activation of an NF-κB-dependent signal. Intriguingly, catalytically inactive mPLK/IRAK inhibits type 1 tumor necrosis factor (TNF) receptor-dependent NF-κB activation. The pathway through which mPLK/IRAK mediates this TNF response is TRADD- and TRAF2-independent. Our data suggest that in addition to its role in IL-1 signaling, mPLK/IRAK is a component of a novel signal transduction pathway through which TNF R1 activates NF-κB-dependent gene expression.

The innate immune response is rapidly activated upon exposure to environmental stimuli and discriminates between self and nonself. The innate immune response may also be involved in determining whether an acquired immune response is required following pathogenic invasion (1). Studies in plants, insects, and mammals have revealed that serine/threonine innate immune kinase family members are components of the innate immune response (2). Serine/threonine innate immune kinase mediate early developmental decisions and, in adult tissue, mediate transactivation of genes whose products are involved in host defense. A Drosophila serine/threonine innate immune kinase family member, Pelle, is a maternal-effect gene that is also required for protection against fungal infections (2, 3). Plant serine/threonine innate immune kinase family members include Pto and Pti-1, which mediate disease resistance in the tomato (4). Two human serine/threonine innate immune kinase family members, interleukin-1 receptor-associated kinases IRAK and IRAK-2 have been linked to signaling through IL-1 receptor family members (5–7).

In response to IL-1 binding, the type I IL-1 receptor recruits the IL-1 receptor accessory protein (IL-1RAcP) and MyD88 (for review, see Ref. 8). The IL-1RAcP and/or MyD88 bind IRAK (9–11) and in a tumor necrosis factor receptor-associated factor-6 (TRAF-6)-dependent manner activate the NF-κB-inducing kinase (NIK; Refs. 12 and 13). NIK promotes activation of the IκB kinase complex (14–18), culminating in activation of NF-κB. IRAK was identified by co-purification with the IL-1RI and was subsequently shown to bind the IL-1RAcP directly (5, 6, 9–11). IRAK-2, like IRAK, is in a complex with IL-1RI and IL-1RAcP and is a component of a transduction pathway downstream of MyD88 (6). IRAK or IRAK-2 overexpression activates the NF-κB-dependent E-selectin gene promoter (6, 11). In response to IL-1 stimulation, the IRAK protein becomes phosphorylated (5, 19). Whether IRAK phosphorylation is an autophosphorylation event or the result of phosphorylation by an independent kinase is not known. Nor is it known whether IRAK phosphorylation is an activation event and/or is required for IL-1-targeted degradation of IRAK by the proteosome (19).

Two types of IRAK and IRAK-2 mutants have been described: mutants that consist of the amino terminus of IRAK or IRAK-2, and for IRAK, a mutant in which a lysine in the putative ATP binding site has been changed to serine (IRAKK293S). Overexpression of the amino terminus of IRAK or IRAK-2 inhibits IL-1-dependent activation of NF-κB activity, thus implicating IRAK and IRAK-2 in IL-1 signaling (6, 7, 11, 20). The effect of IRAKK293S overexpression on IL-1-dependent NF-κB activation has not been reported.

We identified a mouse homologue of Pelle, a Drosophila serine/threonine innate immune kinase family member (21). Based upon sequence identity (5) and chromosomal location (22), mPLK is the mouse homologue of human IRAK. The mPLK protein contains intrinsic protein kinase activity (21). Although IRAK and IRAK-2 share sequence similarity, IRAK-2 lacks key residues thought to be critical for protein kinase

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‡ The abbreviations used are: IL-1, interleukin 1; CAT, chloramphenicol acetyltransferase; LUC, luciferase; mPLK/IRAK, mouse Pelle-like kinase/human interleukin-1 receptor-associated kinase; TNF, tumor necrosis factor; IL-1RAcP, IL-1 receptor accessory protein; ci, catalytically inactive; RI, type I receptor; IP, immunoprecipitation, RIP, receptor-interacting protein; RSV, Roux sarcoma virus; NIK, NF-κB-inducing kinase; TRADD, TNF RI-associated death domain.
kinase catalytic activity is essential in NF-κB signaling and to identify a role for mPLK/IRAK in the TNF RI because it inhibits wild-type mPLK/IRAK activity. We have mPLK/IRAKD358N functions as a dominant-negative allele of mPLK/IRAK. We show here that introduction of a D358N mutation in subdomain VII of mPLK/IRAK abrogates the ability of mPLK/IRAK to induce NF-κB activity. Furthermore, mPLK/IRAKD358N functions as a dominant-negative allele because it inhibits wild-type mPLK/IRAK activity. We have used this mutation to confirm a role for mPLK/IRAK in IL-1 signaling and to identify a role for mPLK/IRAK in the TNF RI signaling pathway.

EXPERIMENTAL PROCEDURES

Plasmid Construction and Plasmids—The IL-8-CAT and IL-8-LUC reporter plasmids were constructed by ligation of the 194–771 bp fragment of the IL-8 promoter immediately upstream of either of the bacterial chloramphenicol acetyltransferase gene (pCAT-Basic, Promega, Madison, WI) or the firefly luciferase cDNA (pGL3, Promega). The indicated mPLK cDNAs were subcloned into a mammalian expression vector that placed them under the control of the cytomegalovirus immediate-early gene promoter (pCMV). The mPLK construct contained the wild-type mPLK cDNA (amino acids 1–771, Ref. 21). The 3′mPLK cDNA encoded amino acids 33–711. The cimPLK mutant contained a point mutation at amino acid 358 (D358N) and, in vitro kinase assays, lacked catalytic activity. The wild-type NIK cDNA, and cNIK encoded NIKKKK429–430 amino acids (13). TRAF2 encoded amino acids 87–501 (26), TRADD encoded the wild-type TRADD cDNA, and ΔTRADD encoded amino acids 102–312 (27). The full-length mPLK cDNA was tagged with a myc epitope at the carboxyl terminus (Invitrogen, San Diego, CA); full-length NIK cDNA contained a carboxyl-terminal FLAG epitope. Epitope-tagged mNIK and NIK constructs were determined to be biochemically active in preliminary studies.

Cell Culture and Transfections—Human embryonic kidney epithelial cells (293 cell line) were maintained in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum, penicillin/streptomycin, and 10% fetal bovine serum. For transient transfection assays, the human embryonic kidney epithelial cells (293 cell line; ATCC) were used. Approximately 2 × 10⁵ cells were plated at a density of 2 × 10⁴ cells/60-mm tissue culture dishes and grown in 5% CO₂ at 37 °C. Plasmid constructs encoding myc-tagged mPLK and FLAG-tagged NIK were transfected into cells 16 h later by calcium phosphate precipitation (28). 48 h later, cell monolayers were harvested.

Cell monolayers were rinsed with phosphate-buffered saline and lysed in 1 ml of immunoprecipitation (IP) lysis buffer (10 m M HEPES, pH 7.4, 150 m M NaCl, 5 m M EDTA, 1% Triton X-100, and complete protease inhibitors). Cell debris was removed by centrifugation, and cell lysates were incubated with the indicated immunoprecipitation antibody. After a 16 h incubation at 4 °C, protein A-Sepharose (10% (v/v) slurry) was added to antibody-containing cell lysates, and reactions were subject to an additional 2-h incubation at 4 °C. Immunocomplexes, collected by centrifugation, were washed two times in IP wash buffer (same as lysis buffer, except [Triton X-100] was 0.1%). Washed material was resuspended in Laemmli buffer, denatured, and subjected to SDS-polyacrylamide gel electrophoresis in 8% reducing polyacrylamide gels. Separated proteins were transferred to Immobilon-P (Millipore) according to manufacturer's specifications.

RESULTS

mPLK/IRAK Activates NF-κB—Treatment of a variety of cell types with inflammatory cytokines, like IL-1α and TNFs, results in transactivation of genes involved in mediating immune and inflammatory responses, including the IL-8 and E-selectin genes (25, 30). Overexpression of human IRAK induces transactivation of the NF-κB-dependent E-selectin gene promoter (11). Therefore we first confirmed that overexpression of mPLK, the mouse IRAK homologue, transactivates an NF-κB-dependent gene promoter in mouse cells. Transient transfection of mouse embryonic fibroblasts with a mammalian expression vector containing the mPLK cDNA led to approximately a 5-fold increase in the activity of the NF-κB-dependent IL-8 gene promoter (Fig. 1A). This level of mPLK-mediated induction of NF-κB activity is comparable with that detected when IRAK is overexpressed in human cells (11). These data are consistent with the proposal that mPLK, like its human homologue, IRAK, lies in a signaling pathway upstream of NF-κB. Substitution of an asparagine for an aspartic acid residue (D358N) in the Mg²⁺-ATP binding site of mPLK created a catalytically inactive mPLK protein (cimPLK). In contrast to results obtained with mPLK, overexpression of cimPLK did not result in transactivation of the IL-8 gene promoter (Fig. 1A). A catalytically active mPLK mutant lacking the first 33 amino acids corresponding to helix 1 of the putative mPLK/IRAK death domain (ΔmPLK; Ref. 21) also did not transactivate the IL-8 gene promoter (Fig. 1A). Thus, mPLK/IRAK catalytic activity and an intact amino-terminal death domain are required for mPLK to induce transactivation of the IL-8 gene promoter. To determine whether cimPLK functions in a dominant-negative manner, mouse embryonic fibroblasts were co-transfected with mPLK and cimPLK. In a dose-dependent manner, cimPLK inhibited the ability of mPLK to induce IL-8 promoter activity (Fig. 1B). Similar results were obtained with ΔmPLK.

The IL-8 gene promoter, like the E-selectin promoter, contains NF-κB and API cis-acting elements (25, 31). Thus, the
observations described above do not define which of these cis-acting elements is activated by mPLK. To verify that the mPLK effect is mediated through NF-κB, the effect of mPLK overexpression on NF-κB-dependent promoters was examined. Like the IL-8 gene promoter, overexpression of mPLK increased E-selectin promoter activity (4-fold; Fig. 1C) and stimulated the activity of a reporter construct under the control of tandem NF-κB sites (see Fig. 3C; Ref. 32). Because the E-selectin and IL-8 promoters also contain binding sites for AP1 family members, we examined whether mPLK affected the activity of an IL-11 promoter construct known to be AP1-dependent (33). Although TNFα treatment led to a 4-fold increase in IL-11 promoter activity, overexpression of mPLK had no effect on IL-11 promoter activity (Fig. 1D). These data demonstrate that mPLK/IRAK lies in a signaling pathway upstream of NF-κB.

The NF-κB-inducing kinase, NIK, is a component of the type I IL-1 receptor (IL-1RI)-signaling cascade leading to NF-κB activation (13). We therefore determined whether induction of the IL-8 gene promoter by mPLK required NIK. Transfection of mouse embryo fibroblasts with NIK led to an approximate 18-fold increase in IL-8 gene promoter activity (Fig. 2). Co-transfection with cinPLK or ΔmPLK had no effect upon NIK-mediated activation of the IL-8 gene promoter (Fig. 2). Malinin et al. (13) describe a dominant-negative allele of NIK, ciNIK (K429A,K430A), capable of blocking IL-1 or TNFα-induced transactivation of the E-selectin gene promoter. However this construct has no effect on p65-induced E-selectin gene promoter activity. We examined whether ciNIK overexpression would affect the ability of mPLK to induce IL-8 gene promoter activity. Results of these assays suggested that mPLK/IRAK-mediated transactivation of the IL-8 gene promoter requires NIK (Fig. 2).

IL-1 Signaling Does Not Require mPLK/IRAK Activity—NIK and mPLK/IRAK are thought to mediate activation of NF-κB through the IL-1RI (11, 13). IRAK-2, which lacks conserved residues in key protein kinase subdomains, can also modulate IL-1-induced NF-κB activation (6). Therefore we determined whether mPLK/IRAK catalytic activity is necessary for IL-1-dependent activation of the IL-8 gene promoter. Overexpression of wild-type mPLK did not block IL-1 induction of IL-8 gene promoter activity (Fig. 3A). Interestingly, neither cinPLK nor ΔmPLK decreased IL-1 induction of IL-8 promoter activity (Fig. 3A). We therefore examined whether the link between IL-1 and mPLK/IRAK activity in mouse embryo fibroblasts was similar to that described for mPLK/IRAK activity in human cells. Overexpression of amino-terminal IRAK (amino acids 1–208 or 1–215) or IRAK-2 (amino acids 1–96) mutants inhibit IL-1-dependent NF-κB activation in human cells (6, 7, 11). Therefore we prepared an mPLK mutant that contained only amino-terminal residues 1–156. Overexpression of the mPLK mutant containing only amino-terminal residues 1–156 in mouse embryo fibroblasts blocked IL-1-dependent NF-κB activation. This result is consistent with that described for
amino-terminal IRAK mutants expressed in human cells (6, 7, 11) and suggests that mPLK activity in mouse cells functions in a manner similar to that proposed for IRAK in human cells. However, when mouse embryo fibroblasts transfected with dominant-negative mPLK/IRAK were treated with IL-1α for varying times, higher levels of IL-8 promoter activity were detected as compared with IL-1α-treated fibroblasts transfected with vector alone (Fig. 3B). To confirm that the enhanced response in IL-1α-treated cultures overexpressing cimPLK was mediated through NF-κB, transient transfection assays were repeated with a reporter construct containing only NF-κB sites ([NF-κB]₃-LUC; Ref. 32). Trace amounts of [NF-κB]₃-LUC activity were detected in cultures transfected with either expression vector minus a cDNA insert or with cimPLK. As was seen for IL-8 promoter activity, a 6-h IL-1 treatment increased [NF-κB]₃-LUC activity 2-fold, and in cultures transfected with cimPLK, IL-1 treatment increased [NF-κB]₃-LUC activity 4-fold. Thus, it appears that NF-κB-dependent signaling through the IL-1RI can be blocked by overexpressing amino-terminal mPLK/IRAK residues or enhanced by overexpressing catalytically inactive mPLK/IRAK.

**Fig. 3.** mPLK activity required for TNF RI-induced NF-κB activity. A, dominant-negative mPLK proteins do not block IL-1α-mediated transactivation of the IL-8 gene promoter. Mouse embryo fibroblasts were co-transfected as described above (Fig. 2B). 16 h before harvest, cultures were treated with recombinant human IL-1α (3000 units; a kind gift from Hoffman-LaRoche Inc., Nutley, N.J.). B, dominant-negative mPLK enhances IL-1α-mediated transactivation of the IL-8 gene promoter. Mouse embryo fibroblasts were transfected with cimPLK (5 µg) and IL-8-LUC (5 µg), and at the indicated times before harvest, cultures were treated with recombinant human IL-1α. C, dominant-negative mPLK blocks TNFα-dependent transactivation of the IL-8 gene promoter. Mouse embryo fibroblasts were cotransfected with 2.5 µg of the IL-8-LUC reporter construct and 5 µg of the indicated plasmid constructs. 16 h before harvest, cultures were treated with recombinant mouse TNFα (100 units; Sigma). The effect of the ciNIK construct on TNFα-induced activation of the IL-8 gene promoter was included as a positive control for monitoring inhibitory activity.
fibroblasts transfected with mPLK are treated with TNFα, no additional increase in IL-8 gene promoter activity is observed (Fig. 3C). However, when mouse embryo fibroblasts are transfected with cimPLK or ΔmPLK, TNFα-induced transactivation of the IL-8 gene promoter is decreased (Fig. 3C). The cimPLK- and ΔmPLK-mediated decrease in TNFα-induced activation of the IL-8 gene promoter was dose-dependent; however, overexpression of either cimPLK or ΔmPLK did not completely abrogate a TNF-dependent signal. Analysis of cells derived from TNF RI and TNF RII nulligenic animals revealed that TNF-induced NF-κB activity is mediated solely through TNF RI (36). Consistent with this observation, cimPLK decreased IL-8 promoter activity in mouse fibroblasts treated with human TNFα, which on mouse cells signals exclusively through TNF RI (37).
Taken together, these data demonstrate that mPLK/IRAK is a component of a TNF RI signaling pathway and provide further evidence for mPLK/IRAK as a component of the IL-1-signaling pathway.

IL-1-mediated activation of NF-κB-dependent promoters is inhibited by a mutated version of TRAF6 (11, 12). We found that dominant-inhibitory TRAF6 (ΔTRAF6289–522) inhibits IL-1-dependent activation of the IL-8 gene promoter 10-fold but did not significantly inhibit TNF- or mPLK-dependent activation (1.4-fold decrease under either condition). These results support those of Cao and co-workers (11, 12), whereby similar amounts of the dominant-inhibitory TRAF6 reduced an IL-1-dependent signal 10-fold and reduced TNF- and the IRAK-dependent signals 1.5-fold.

TNF-induced NF-κB activity is mediated in part by TNF RI-associated death domain (TRADD) and two TRADD-recruited proteins: TNF receptor associated factor-2 (TRAF2) and the TNF receptor-interacting protein (RIP; Refs. 38–41). To determine whether mPLK/IRAK is required for TRADD/TRAF2/RIP-mediated activation of the IL-8 gene promoter, the effects of cimPLK and ΔmPLK on TRADD and TRAF2-induced IL-8 gene promoter activity were measured. Neither cimPLK nor ΔmPLK blocked the ability of TRADD (Fig. 4B) or TRAF2 (Fig. 4A) to induce activation of the IL-8 gene promoter.

We next determined if TRADD or TRAF2 were required for mPLK/IRAK-mediated activation of NF-κB. In contrast to mPLK, which does not transactivate AP1-dependent promoters (Fig. 1D), TRAF2 activates AP1 as well as NF-κB-dependent promoters (42). The IL-8 gene promoter, like the E-selectin dependent signal 10-fold and reduced TNF- and the IRAK-dependent signals 1.5-fold.

TNF RI signaling in part is thought to be mediated by the recruitment of death domain-containing proteins, which bind to the carboxy-terminal death domain in TNF RI (13, 34, 35). Recruitment of death domain-containing proteins, which bind ing pathway.

that mPLK/IRAK lies in a TRADD/TRAF2-independent signal-

D pathway. IRAK-2 has been described as a relative of IRAK that contains a similar motif (WHL; Refs. 5 and 21), we postulated that the mPLK/IRAK and NIK proteins may complex. To test this hypothesis, human embryonic kidney epithelial cells (293 cell line) were co-transfected with plasmids encoding myc-tagged mPLK and FLAG-tagged NIK. Myc or FLAG antisera were used to immunoprecipitate proteins from cell lysates. Immunoprecipitated proteins were subjected to SDS-polyacrylamide gel electrophoresis, followed by Western blot analysis. mPLK and NIK were detected in immunoprecipitates generated with either the FLAG or the myc antisera (Fig. 6, A and B). However, neither mPLK nor NIK were in immunocomplexes prepared with an unrelated mouse IgG (Fig. 6, A and B, lanes 1 and 2, respectively). These data indicate that mPLK/IRAK and NIK can complex in cells.

DISCUSSION

mPLK/IRAK has been linked to signaling through IL-1 receptor family members (5–7) and has been shown to have protein kinase activity in vitro (21). However, the importance of mPLK/IRAK protein kinase activity in signaling has not been addressed. Frequently, mutations within the ATP binding site of protein kinases not only encode nonfunctional protein kinases but also interfere with the function of the wild-type protein (23). Thus, this mutation can be described as a dominant negative allele of mPLK/IRAK (44) and will be useful for further dissection of mPLK/IRAK function. We report here that an mPLK/IRAK mutant lacking catalytic activity (D358N) was unable to induce the activity of NF-κB-dependent promoters. Furthermore, the D358N mPLK/IRAK mutant decreased the ability of wild-type mPLK/IRAK to activate an NF-κB-dependent promoter in a dose-dependent fashion.

Interestingly, overexpression of cimPLK does not inhibit the ability of IL-1 to induce the activity of an NF-κB-dependent promoter. In fact, overexpression of cimPLK enhances an IL-1-dependent signal. This result suggests that mPLK/IRAK catalytic activity is not required for its role in the IL-1-signaling pathway. IRAK-2 has been described as a relative of IRAK that can also enhance an IL-1 signal (6). IRAK-2 is quite similar to IRAK; however, it lacks key residues in several of the highly conserved protein kinase subdomains and is unlikely to be catalytically active. Thus, cimPLK may enhance an IL-1 signal by mimicking IRAK-2. In this context, independent of catalytic activity, IRAK2 and/or cimPLK may subserve a scaffolding function and facilitate formation of signaling complexes. Alternatively, in response to IL-1, phosphatidylinositol 3-kinase activity is also increased (45), which, independent of IRAK/mPLK, may effect changes in NF-κB-dependent signaling.
Enhancement of the IL-1-dependent signal in the presence of cimPLK/IRAK suggests mPLK/IRAK catalytic activity may negatively regulate IL-1-initiated signaling. mPLK/IRAK mutants that inhibit or enhance an IL-1-dependent signal may have therapeutic utility. Clearly, identification of targets that can be used to block IL-1-dependent signaling is important for down-regulating inflammatory responses. As important, however, may be the identification of targets that enhance/activate an inflammatory response in an otherwise immunocompromised host.

TRAF6 has been linked to IL-1 and mPLK/IRAK signaling (11, 12). Although overexpression of a dominant-inhibitory TRAF6 mutant decreases IL-1 signaling, this mutant has a weaker inhibitory effect on mPLK/IRAK. In fact, mutant TRAF6 interferes with IRAK/mPLK and TNF similarly. Our own observations have confirmed these findings. Thus the inhibitory effect of the TRAF6 mutant is much more robust in the context of the IL-1-signaling pathway than in the mPLK/IRAK or TNF RI signaling pathways. Interestingly, TRAF6 was recently shown to complex with and affect signaling through the low affinity nerve growth factor receptor (46), another member of the TNF receptor superfamily (47).

In addition to a protein kinase catalytic domain, mPLK/IRAK also contains an amino-terminal domain that resembles the death domain of proteins linked to TNF RI signaling (35). Indeed, endogenous mPLK and TNF RI proteins can be found complexed. Moreover, overexpression of cimPLK decreases the ability of TNF to induce the activity of NF-κB-dependent promoters. Thus, in contrast to the IL-1-signaling pathway, the catalytic activity of mPLK/IRAK is critical for TNF signaling and suggests that mPLK/IRAK substrates are likely to be components of the TNF signaling pathway.

Overexpression of catalytically inactive NIK, the protein kinase that is believed to phosphorylate IκB kinases (14–18), blocks the activity of mPLK/IRAK. However, overexpression of wild-type NIK in the presence of catalytically inactive mPLK results in activation of NF-κB-dependent promoters. These data suggest that mPLK/IRAK is upstream of NIK and suggests a TNF signaling pathway in which mPLK/IRAK is important for transmitting a signal from TNF RI to NIK. In support of this hypothesis, mPLK/IRAK protein can complex with NIK. mPLK/IRAK signaling is independent of the TNF-signaling molecules TRAF2 and TRADD, indicating that mPLK/IRAK represents a previously undiscovered TNF RI signaling pathway. The latter observation is consistent with the analysis of TRAF2 nulligenic animals, which revealed that TNF RI can mediate NF-κB activation in a TRAF2-independent manner (43, 48). These results suggest a model whereby TNF binding to TNF RI leads to activation of mPLK/IRAK protein kinase activity and the subsequent phosphorylation of mPLK/IRAK substrates, leading to the activation of NF-κB.

Our data places mPLK/IRAK in the TNF RI signaling pathway and confirms a role for mPLK/IRAK in the IL-1-signaling pathway. These data also suggest that the regulation of mPLK/IRAK activity may be more complicated than previously appreciated. In the IL-1 pathway, mPLK/IRAK catalytic activity is not required and, thus, is similar to the role of RIP in TNF signaling. Although cells lacking RIP are defective in TNF signaling, the defect can be reversed by expression of wild-type or catalytically inactive RIP (40, 49). How RIP or mPLK/IRAK may transduce these effects is unclear. Our data suggests that TNF and IL-1 signaling may be coordinated at the level of mPLK/IRAK. In response to IL-1, the mPLK/IRAK protein is targeted for degradation (19). Interestingly, CD30 and TNF RI potentiate TNF RI-induced apoptosis by inducing the degradation of TRAF2 (50). It thus seems possible that mediation of cross-talk between the IL-1 and TNF RI signaling pathways may occur through the targeted degradation of mPLK/IRAK.

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