Toll-like receptor (TLR) signaling is known to involve interleukin-1 receptor-associated kinases (IRAKs), however the particular role of IRAK-2 has remained unclear. Further, although IRAK-1 was originally thought to be central for the TLR-NFκB signaling axis, recent data have shown that it is dispensable for NFκB activation for some TLRs and demonstrated an alternative role for it in interferon regulatory factor activation. Here we show that IRAK-2 is critical for the TLR-mediated NFκB activation pathway. The poxviral TLR antagonist A52 inhibited NFκB activation by TLR2, -3, -4, -5, -7, and -9 ligands, via its interaction with IRAK-2, while not affecting interferon regulatory factor activation. Knockdown of IRAK-2 expression by small interfering RNA suppressed TLR3, TLR4, and TLR8 signaling to NFκB in human cell lines, and importantly, TLR4-mediated chemokine production in primary human cells. IRAK-2 usage by different TLRs was distinct, because it acted downstream of the TLR adaptors MyD88 and Mal but upstream of TRIF. Expression of IRAK-2, but not IRAK-1, led to TRAF6 ubiquitination, an event critical for NFκB activation. Further, IRAK-2 loss-of-function mutants, which could not activate NFκB, were incapable of promoting TRAF6 ubiquitination. Thus we propose that IRAK-2 plays a more central role than IRAK-1 in TLR signaling to NFκB.

Toll-like receptors (TLRs) recognize specific pathogen-associated molecular patterns found on infectious agents (1). TLRs are part of the larger IL-1R/TLR superfamily, defined by the presence of a cytoplasmic Toll-IL-1R-resistance (TIR) signaling domain, which also includes the IL-1, IL-18, and IL-33 receptors. Upon engagement of distinct TLRs by specific pathogen-associated molecular patterns, such as bacterial lipoprotein (for TLR2), viral dsRNA (for TLR3), or LPS (for TLR4), intracellular signaling cascades mediate activation of transcription factors such as NFκB and interferon regulatory factors (IRFs) leading to gene induction and the production of pro-inflammatory cytokines, chemokines and interferons.

Upon activation, IL-1R and all of the TLRs, excluding only TLR3, recruit the TIR domain-containing adaptor molecule myeloid differentiation factor 88 (MyD88) through a TIR-TIR homotypic protein interaction, leading to NFκB activation (2). For TLR2 and TLR4, the TIR adaptor Mal (3) recruits MyD88 to the receptor complex (4). TLR3 utilizes another TIR adaptor known as TIR domain-containing adaptor inducing interferon β (TRIF) (5, 6). TLR4 can also signal through TRIF, via a bridging adaptor called TRIF-related adaptor molecule (TRAM), resulting in a delayed MyD88-independent NFκB response (7). Formation of the TLR-adaptor complex leads to the recruitment of interleukin-1 receptor associated kinases (IRAKs) (8). Four IRAKs have been identified namely IRAK-1, IRAK-2, IRAK-M, and IRAK-4. Both IRAK-1 and IRAK-4 are active Ser/Thr kinases, and phosphorylation of IRAK-1 by IRAK-4 is crucial for IRAK-1 activation during TLR signaling (9, 10). Mice lacking IRAK-4 are completely resistant to LPS treatment and, furthermore, display attenuated IL-1R-, TLR2-, TLR3-, and TLR9-induced cytokine responses (11). Conversely, although IRAK-1 was originally thought to be critical for TLR-induced NFκB activation, IRAK-1-deficient mice show only partial defects in IL-1-, IL-18-, and LPS-induced signaling (12–14). IRAK-M on the other hand has been identified as an inhibitor of TLR signaling (15).

Previous studies have implicated IRAK-2 in IL-1R and TLR4 signaling by showing that IRAK-2 can associate with MyD88 and Mal (3, 16). Furthermore, the poxvirus protein A52, which interacts with both IRAK-2 and TRAF6, can inhibit TLR-induced NFκB activation (16, 17). However, the extent of the role of IRAK-2 in TLR signaling and its mechanism of action remain to be established. Here we present evidence that A52 inhibits NFκB solely via its interaction with IRAK-2 and not TRAF6, and that IRAK-2 is required for all TLR-mediated pathways to NFκB. We show that IRAK-2, but not IRAK-1 causes TRAF6 ubiquitination, a critical step in the activation of NFκB by TLRs. Thus we propose that IRAK-2 has a more fundamental role than IRAK-1, in the TLR-NFκB axis.

**EXPERIMENTAL PROCEDURES**

Expression Vectors—Generation of the A52 and ΔA52 expression vectors has been described previously (16, 17). The source of other plasmids was: IRAK-2-Myc, kIRAK-2-Myc, and AU1-MyD88 (M. Muzio, Mario Negri Institute, Milan, Italy),
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TLR3-FLAG (D. Golenbock, University of Massachusetts Medical School, Worcester, MA), CD4-TLR4 (R. Medzhitov, Yale University, New Haven, CT), IRAK-1 and FLAG-TRAF6 (Tularik Inc., San Francisco, CA), Mal, TRAM, and retinoic acid-inducible gene-I (K. Fitzgerald, University of Massachusetts Medical School, Worcester, MA), TRIF (C. Basler, Mount Sinai School of Medicine, New York, NY), and HA-ubiquitin (A. Mansell, Monash University, Melbourne, Australia).

Antibodies and Reagents—Antibodies used were: anti-Myc mAb, anti-β-actin mAb, and anti-FLAG mAb (Sigma); anti-HA mAb ( Covance); anti-IRAK-1 mAb and anti-TRAF6 polyclonal Ab (Santa Cruz Biotechnology, Santa Cruz, CA); and anti-IRAK-2 polyclonal Ab (Upstate Biotechnologies). Human recombinant IL-1α was from NCI, National Institutes of Health (Frederick, MD). TLR agonists used were synthetic tripalmitoyl lipopeptide Pam3Cys-Ser-(Lys)4 (Pam3CSK4, InvivoGen), 2-kDa macrophage-activating lipopeptide (MALP-2; Alexis), poly(I:C) (Amersham Biosciences), LPS (Alexis), flagellin (a gift from A. Gewirtz, Emory University, Atlanta, GA), R-848 and CL075 (InvivoGen), and phosphothioate CpG (Sigma).

Reporter Gene Assays—NFκB, p38 MAP kinase, IRF3, IRF5, and IRF7 activation as well as IL-8 promoter induction were measured by reporter gene assay. HEK293 or RAW264.7 cells were seeded into 96-well plates at 2×10⁴ and 4×10⁴ cells per well, respectively, and co-transfected with luciferase reporter gene plasmids and expression plasmids 24 h later using the Genejuice® transfection reagent (Novagen) as described previously (20, 21). For all assays, 20 ng of pHRL-TK reporter (Promega) per transfection was included to normalize data for transfection efficiency, and total DNA concentrations were kept constant at 230 ng (for HEK293) or 200 ng (for RAW264.7) by the addition of pcDNA3.1 empty vector (Invitrogen). For measurement of NFκB activity, 60 ng of a κB-luciferase reporter gene was used. The PathDetect System (Stratagene) was used to assess p38 MAP kinase activity with 0.25 ng of a CHO-Gal4 fusion protein expression vector co-transfected with 60 ng of the pFR-luciferase reporter plasmid. IRF activity was determined by co-transfection of 3 ng of either IRF3-, IRF5- or IRF7-Gal4 fusion protein expression vectors as required in conjunction with the pFR-luciferase reporter plasmid (60 ng). Cells were stimulated with TLR agonists for 6 h prior to lysis and measurement of luciferase activity 24 h following transfection. All transfections were done in triplicate, and data are expressed as –fold induction (mean ± S.D.) relative to control levels for a representative experiment of a minimum of three separate experiments.

ELISA—IL-8 was measured by ELISA (20). Each experiment was done in triplicate, and data are expressed as picograms/ml IL8 (mean ± S.D.) for a representative of at least three independent experiments.

siRNA Gene Silencing—siRNA duplexes targeting the human IRAK-2 gene (Qiagen) targeted the following sites: IRAK-2A, 5’-CAGCAACGTCAAGAGCTCTA; IRAK-2B, 5’-CCA-GATCATCCTGACTGGAA. To confirm knockdown of IRAK-2-Myc expression, HEK293 cells (3×10⁵ cells) were transfected with siRNA duplexes using Lipofectamine 2000 ( Invitrogen), followed by transfection with IRAK-2-Myc and a second siRNA transfection 24 h later. After a further 24 h, cell lysates were prepared and analyzed by SDS-PAGE and immunoblotting. For reporter gene assays, HEK293 cells in 96-well plates (3×10⁴ cells per well) were transfected with siRNA duplexes and then co-transfected with reporter constructs and a second dose of siRNA 24 h later prior to stimulation with 25 μg/ml poly(I:C) or 100 ng/ml LPS for 6 h. For siRNA experiments in primary human cells, peripheral blood mononuclear cells were isolated from healthy donor whole blood using Lymphoprep reagent (Axis-shield), seeded in 96-well plates (8×10⁴ cells per well) at 37°C for 2 h, and transfected with siRNA as above. A second siRNA transfection was delivered 24 h later, and cells were treated for 24 h with 10 ng/ml LPS 48 h following initial transfection. Cell supernatants were harvested and IL-8 cytokine production analyzed by ELISA.

Co-immunoprecipitation and Immunoblotting—Proteins were co-immunoprecipitated from transfected HEK293T cells as previously described (20). For immunoprecipitation of ubiquitinated proteins, lysis buffer was supplemented with 10 μM iodoacetamide. Target proteins were immunoprecipitated by incubating 400 μl of lysate with the relevant antibody, pre-coupled to protein A- or G-Sepharose beads, for 2 h at 4°C. Immune complexes were analyzed by SDS-PAGE and immunoblotting.

Site-directed Mutagenesis of IRAK-2—Point mutations were introduced into the IRAK-2 gene sequence using the QuikChange™ site-directed mutagenesis kit (Stratagene) according to manufacturer’s instructions.

RESULTS

A52 Specifically Targets IRAK-2 to Inhibit Signal Transduction from TLRs to NFκB—Previously we showed that poxvirus protein A52 could inhibit NFκB activation by TLR2, -3, -4, or -5 in human HEK293 cells, and associate with both TRAF6 and IRAK-2 (18). Here, we extended this observation to demonstrate that A52 expression antagonized ligand-induced NFκB activation through all TLRs tested, including TRL7 and TRL9, in murine macrophage RAW264.7 cells (Fig. 1a). Further A52 substantially reduced MALP-2- and R848-mediated NFκB induction in HEK293 cells stably expressing TLR2 and TLR8, respectively (data not shown). Thus, it appeared that A52 was capable of inhibiting all TLR pathways to NFκB, both in human and murine cells. We therefore next determined whether the ability of A52 to inhibit NFκB depended on its interaction with TRAF6 and/or IRAK-2. Previously, the A52-TRAF6 interaction has been shown to induce MAP kinase activation, whereas ΔA52, a truncated A52 protein (comprising amino acids 1–144 of the 190-residue protein) could no longer associate with TRAF6 nor induce MAP kinase activation (19). Because ΔA52 could still interact with IRAK-2, it provided a useful tool to investigate IRAK-2 involvement in NFκB activation. Fig. 1 (b–d) shows that A52 substantially reduced the induction of an NFκB-dependent reporter gene stimulated by exogenous treatment of IL-1R-expressing cells with IL-1α, co-expression of TLR3 and simultaneous poly(I:C) treatment or introduction of a constitutively active CD4-TLR4 chimera and compellingly, ΔA52 was as powerful a suppressor of NFκB activation via IL-1R, TLR3, and TLR4 as wild-type A52. Thus the interaction
of A52 with TRAF6 is dispensable for inhibition of IL-1R- and TLR-induced NFκB activation, suggesting that it is via its association with IRAK-2 that A52 can potently antagonize TLR signaling to NFκB, thus pointing to IRAK-2 as a critical downstream component of these signaling cascades.

**IRAK-2 Induces NFκB and p38 MAP Kinase**—As previously shown (16) IRAK-2 expression, like IRAK-1, led to NFκB activation (Fig. 1e). To determine the effect of IRAK-2 on p38 MAP kinase activation, another downstream event in TLR signal-
trigger IRF3 or IRF5 activation in these experiments (Fig. 1, g and h). Similarly, IRF7 was not stimulated by IRAK2 (data not shown).

Inhibition of IRAK-2 Suppresses TLR-induced NFκB Activation—We next took a more direct approach to investigate the potential role of IRAK-2 in the TLR-NFκB signaling axis. An IRAK-2-truncated protein (with amino acids 97–590, termed kIRAK-2) has been shown to act as a dominant negative, abolishing the induction of NFκB by the IL-1R (16), and here we also found that kIRAK-2 inhibited ligand-induced TLR3-, TLR4-, and TLR8-mediated NFκB activation, whereas NFκB activation induced through ectopically expressed retinoic acid-inducible gene-1 was not blocked (Fig. 3a). Like TLR3, retinoic acid-inducible gene-1 is a viral RNA pattern recognition receptor that mediates NFκB activation (28).

To obtain independent evidence for a role in IRAK-2 in signal transduction from TLR3, TLR4, and TLR8 to NFκB, a, HEK293 cells stably expressing TLR3 (for poly(I:C)), TLR4 (for LPS), and TLR8 (for CL075) or transfected with 50 ng of retinoic acid-inducible gene-I expression plasmid were co-transfected with pcDNA3.1 (black bars), 30 ng of kIRAK-2 (gray bars), or 100 ng of kitIRAK2 (white bars). Cells were treated with 25 μg/ml poly(I:C), 100 ng/ml LPS, or 5 μg/ml CL075 where indicated for 6 h prior to assaying NFκB reporter gene activity. b, HEK293 cells were transfected with pcDNA3.1 (EV) or IRAK-2-Myc and 100 pmol of either control non-silencing siRNA (con) or siRNA targeting the IRAK-2 gene (IRAK2A or IRAK2B), and cell lysates were assayed for IRAK-2-Myc expression by immunoblotting with anti-Myc Ab (upper panel). Samples were immunoblotted with anti-β-actin Ab to confirm equal protein loading (lower panel). In c–f, HEK293 (c), HEK293-TLR3 (d), HEK293-TLR4 (e), and HEK293-TLR8 (f) cells were transfected with 20 pmol of non-silencing control siRNA, IRAK-2A or IRAK-2B siRNAs targeting IRAK-2 expression and, where indicated, with pcDNA3.1 (control) or 30 ng of IRAK-2. Cells were stimulated with 25 μg/ml poly(I:C) (d), 100 ng/ml LPS (e), or 5 μg/ml CL075 (f) for 6 h prior to NFκB reporter gene assay. Data in a and c–f are fold induction (mean ± S.D. of triplicates) relative to control levels and are representative of at least three independent experiments. g, human peripheral blood mononuclear cells were transfected with 2.5 pmol of either non-silencing control siRNA or IRAK-2A siRNA. Cells were stimulated with 10 ng/ml LPS for 24 h, and IL-8 production in cell supernatants was assayed by ELISA. The experiment shown is representative of data obtained from three independent healthy donors (error bars = ±S.D.).

FIGURE 3. IRAK-2 is required for signal transduction from TLR3, TLR4, and TLR8 to NFκB.
Role for IRAK-2 in TLR Signaling

MyD88-induced NFκB activity by almost 50%. NFκB activity induced by Mal was potently blocked in cells treated with both siRNAs (Fig. 4b). These data are consistent with previous proposals of IRAK-2 acting downstream of MyD88 (16) and Mal (3). Surprisingly, although NFκB activation by poly(I:C)/TLR3 was sensitive to IRAK-2 siRNAs (Fig. 3d), no inhibition of TRIF-induced NFκB activation was observed (Fig. 4d). These data suggested alternative usage of IRAK-2 in the MyD88- and TRIF-dependent pathways to NFκB activation. The fact that TLR3 signals independently of MyD88 and Mal and solely utilizes TRIF, yet has a requirement for IRAK-2, suggested that IRAK-2 might be recruited into the TLR3 signaling complex upstream of TRIF.

IRAK-2 Associates with TLR3—Because IRAK-2 can associate with Mal (3) and form a complex with the IL-1R (16), we reasoned that IRAK-2 could interact with TIR domain-containing proteins, in contrast to IRAK-1, which accesses IL-1R-TLR complexes via a death domain interaction with MyD88. We therefore tested whether IRAK-2 could associate with the TLR3 receptor complex. Fig. 4e demonstrates by co-immunoprecipitation an association between ectopically expressed TLR3 and IRAK-2. This complex was detectable in the absence of poly(I:C) treatment, and the intensity of the receptor-IRAK-2 interaction was unchanged following stimulation of TLR3 by poly(I:C). Co-immunoprecipitation experiments carried out with ectopically expressed TLR3 and endogenous IRAK-2 confirmed the ability of IRAK-2 to physically associate with TLR3 (Fig. 4f). Again, the IRAK-2-TLR3 receptor complex was detectable in the absence of poly(I:C) treatment, which may reflect the strength of the association between these two molecules.

IRAK-2 Activates NFκB by Stimulating TRAF6 Ubiquination—IRAK-2 can also interact with the non-TIR domain-containing protein TRAF6 (16), which is an important downstream effector molecule for transcription factor and MAP kinase activation by IL-1 and TLRs. Fig. 5a shows a sequence alignment of mammalian IRAK-2 orthologs indicating the position of two putative consensus TRAF6 binding motifs within IRAK-2, as previously noted by Ye et al. (30). These motifs were mutated to explore the importance of TRAF6 as an effector of IRAK-2 function. The central Glu residues of both sites were mutated...
concurrently to Ala, because mutation of this residue within the single TRAF6 binding motif of CD40 severely impaired its function (30). Fig. 5b shows that this mutant, IRAK-2DM, was no longer able to activate NFκB (left panel), p38 MAP kinase activation (middle panel), or to induce the IL-8 promoter (right panel). Thus, mutation of the two putative TRAF6 binding motifs within IRAK-2 completely silenced all IRAK-2 functions tested suggesting that TRAF6 is a critical effector for IRAK-2-induced NFκB and MAP kinase activation. The relative contribution of each TRAF6 binding motif to IRAK-2 function was examined by mutating each central Glu independently to generate two distinct mutants, E528A and E559A. IRAK-2E559A induced signal transduction pathways as potently as the wild-type protein (Fig. 5, compare c to b). Surprisingly, IRAK-2E528A was entirely inert, unable to induce NFκB or p38 MAP kinase activation, or to up-regulate the IL-8 promoter (Fig. 5c). Both mutant proteins were expressed at comparable levels (Fig. 5d). Hence a single amino acid (Glu-528), in the first TRAF6 binding motif is essential for IRAK-2 function, whereas the second putative TRAF6 binding motif does not appear to be important for IRAK-2 signaling to NFκB or p38 MAP kinase activation.

Because mutation of the central Glu residue within the TRAF6 binding motif of CD40 abolished its ability to interact with TRAF6 in an in vitro binding assay, thus accounting for the ensuing loss of activity (30), we compared the ability of the mutants to interact with TRAF6. Surprisingly, when IRAK-2E528A was expressed in cells it was still capable of interacting with TRAF6 (Fig. 5d, left panels). As expected, the functionally active IRAK-2E559A also associated with TRAF6 (Fig. 5d, mid-
Role for IRAK-2 in TLR Signaling

Although identified 10 years ago (16), the exact role of IRAK-2 in TLR signaling has remained unclear, as has the extent of its involvement in different TLR pathways. Here, we have exploited the ability of the poxvirus protein A52 to inhibit TLR signaling to implicate IRAK-2 as having a central role in NFκB activation. Like other known virulence factors, A52 seems to have multiple effects on the host immune response, and the availability of an A52 mutant, which only interacted with IRAK-2 and not its other target TRAF6, allowed us to map the inhibitory effect of A52 on the host immune response, and the availability of an A52 mutant, which only interacted with IRAK-2 and not its other target TRAF6, allowed us to map the inhibitory effect of A52 on the host immune response. Although it has been assumed that IRAK1 causes TRAF6 polyubiquitination, here comparison of IRAK-2 to IRAK-1 in the ubiquitination assay revealed that IRAK-1 lacked the ability of IRAK-2 to initiate TRAF6 polyubiquitination (compare lane 4 to lane 6; Fig. 5f, upper panel). Critically, given the essential requirement for this key step in the induction of NFκB in response to TLR signal transduction, this functional difference between IRAK-1 and IRAK-2 provides strong evidence for a more central role for IRAK-2 in TLR signaling to NFκB compared with IRAK-1.

DISCUSSION

Although identified 10 years ago (16), the exact role of IRAK-2 in TLR signaling has remained unclear, as has the extent of its involvement in different TLR pathways. Here, we have exploited the ability of the poxvirus protein A52 to inhibit TLR signaling to implicate IRAK-2 as having a central role in NFκB activation. Like other known virulence factors, A52 seems to have multiple effects on the host immune response, and the availability of an A52 mutant, which only interacted with IRAK-2 and not its other target TRAF6, allowed us to map the inhibitory effect of A52 on the host immune response. Although it has been assumed that IRAK1 causes TRAF6 polyubiquitination, here comparison of IRAK-2 to IRAK-1 in the ubiquitination assay revealed that IRAK-1 lacked the ability of IRAK-2 to initiate TRAF6 polyubiquitination (compare lane 4 to lane 6; Fig. 5f, upper panel). Critically, given the essential requirement for this key step in the induction of NFκB in response to TLR signal transduction, this functional difference between IRAK-1 and IRAK-2 provides strong evidence for a more central role for IRAK-2 in TLR signaling to NFκB compared with IRAK-1.
Mal-dependent pathways to NFκB, IRAK-2 seems to act upstream of the TRIF-dependent pathway (Fig. 4, a–c), consistent with the interaction of IRAK-2 with TLR3. A model for IRAK2 involvement in TLR signaling pathways to NFκB is shown in Fig. 6. It is currently unclear how exactly TRIF is involved in IRAK-2-mediated NFκB activation by TLR3. Given that, in the absence of TRIF, signaling to NFκB from TLR3 is disrupted (6, 33), it is unlikely that IRAK-2 controls a dominant TLR3 pathway to NFκB independently of TRIF. However, because these studies utilized knock-out mouse models, it remains to be seen whether TRIF functions identically in TLR3 signaling in humans.

Recent studies have questioned the originally proposed central role of IRAK-1 in NFκB activation. For TLR9 in dendritic cells, IRAK-1 was dispensable for NFκB activation and pro-inflammatory cytokine production (34). TLR9 is thought to signal in a similar manner to TLR8, and herein, we show that IRAK2 siRNA inhibited TLR8-mediated NFκB activation. Further, alternative NFκB-independent functions for IRAK-1 have emerged, such as TLR4-mediated STAT3 activation and up-regulation of the NFκB-independent gene, IL-10 (35). In that study, absence of IRAK-1 had no effect on LPS induction of the NFκB-dependent pro-inflammatory cytokine, IL-1β. In our study, IRAK-2 siRNA inhibited LPS-induced NFκB activation, and, in primary human cells, LPS stimulated production of the NFκB-dependent chemokine IL-8. Moreover, IRF7 has recently been identified as a target of IRAK-1 kinase activity (34). The formation of an IRF7-IRAK-1 complex followed by IRAK-1-mediated phosphorylation of IRF7 appeared to be a prerequisite for interferon-α induction by TLR7 and TLR9. In contrast, the lack of any effect of A52 or IRAK2 expression on IRF3, -5, and -7 activation suggests that IRAK-2 has no role in IRF activation. Hence IRAK-1, although involved in some pathways to NFκB, may be more fundamentally important for TLR signaling to IRFs. Here, the observation that IRAK-2 but not IRAK-1 could trigger TRAF6 ubiquitination provides direct evidence for the importance of IRAK-2 over IRAK-1 in TLR-mediated NFκB activation, because the autoubiquitinating E3 ligase activity of TRAF6 is essential for IL-1R/TRL signaling to NFκB (36, 37), although how exactly this activity of TRAF6 is turned on by IRAK-2 is yet to be established. Although it had been assumed that IRAK-1 could stimulate TRAF6 ubiquitination, a recent study demonstrated that the Epstein-Barr virus protein LMP1 could cause TRAF6 polyubiquitination in the absence of IRAK-1 (38). Thus we have defined a dichotomy in IRAK-1 and IRAK-2 function, which suggests that these proteins are not simply functionally redundant but rather fulfill separate roles in TLR signaling.

Despite two potential TRAF6-binding motifs being previously identified in IRAK-2 (30), their functional significance had not been tested until now. The site containing Glu-528 is conserved among mammalian IRAK-2 orthologs, whereas the second putative TRAF6 binding site containing residue Glu-559 is not, consistent with the functional relevance of the Glu-528-containing site, which when mutated abolished the ability of IRAK-2 expression to induce NFκB and MAP kinase activation and IL-8 promoter induction. The fact that IRAK-2E528A still binds to TRAF6 and yet cannot activate NFκB suggests that Glu-528 is in fact critical for signal transduction, and consistent with this, IRAK-2E528A no longer induced TRAF6 polyubiquitination.

In conclusion, we have demonstrated that IRAK-2 has a central role in TLR signaling pathways to NFκB activation, and in contrast to IRAK-1, is likely utilized by all TLRs for this purpose. IRAK-2 can interact with at least one TLR, namely TLR3, and TRAF6 is a critical downstream mediator of IRAK-2 function. Given that IRAK-2 is unlikely to be involved in IRF activation, selectively targeting IRAK-2 may be a useful therapeutic strategy to block pro-inflammatory TLR signals via NFκB, while preserving IRF-regulated pathways.

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