The Journal of Immunology

Cutting Edge: Expression of IL-1 Receptor-Associated Kinase-4 (IRAK-4) Proteins with Mutations Identified in a Patient with Recurrent Bacterial Infections Alters Normal IRAK-4 Interaction with Components of the IL-1 Receptor Complex

Andrei E. Medvedev, Karen Thomas, Agnes Awomoyi, Douglas B. Kuhns, John I. Gallin, Xiaoxia Li and Stefanie N. Vogel

J Immunol 2005; 174:6587-6591; ;
doi: 10.4049/jimmunol.174.11.6587
http://www.jimmunol.org/content/174/11/6587

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852

Print ISSN: 0022-1767 Online ISSN: 1550-6606.

THE JOURNAL OF IMMUNOLOGY

CUTTING EDGE

Cutting Edge: Expression of IL-1 Receptor-Associated Kinase-4 (IRAK-4) Proteins with Mutations Identified in a Patient with Recurrent Bacterial Infections Alters Normal IRAK-4 Interaction with Components of the IL-1 Receptor Complex[1]

Andrei E. Medvedev, Karen Thomas,* Agnes Awomoyi,* Douglas B. Kuhns,† John I. Gallin,‡ Xiaoxia Li,§ and Stefanie N. Vogel[2]**

***In a patient with recurrent bacterial infections and profound hyporesponsiveness to LPS and IL-1, we previously identified two mutations in IL-1R-associated kinase-4 (IRAK-4) that encoded proteins with truncated kinase domains. Overexpression of either of these mutant IRAK-4 variants in HEK293 cells failed to activate endogenous IRAK-1 and suppressed IL-1-induced IRAK-1 kinase activity, in contrast to wild-type (WT) IRAK-4. In this study, interactions of WT and mutant IRAK-4 species with IL-1R, IRAK-1, and MyD88 in HEK293 transfectants were compared. IL-1 induced a strong interaction among the IL-1R, activated IRAK-1, MyD88, and WT, but not mutant, IRAK-4. Truncated IRAK-4 proteins constitutively interacted more strongly with MyD88 and blunted IL-1-induced recruitment of IRAK-1 and MyD88 to the IL-1R. Thus, decreased IL-1-induced association of IRAK-1 and MyD88 with the IL-1RI may result from sequestration of cytoplasmic MyD88 by IRAK-4 mutant proteins. Therefore, mimetics of these truncated IRAK-4 proteins may represent a novel approach to mitigating hyperinflammatory states. The Journal of Immunology,* 2005, 174: 6587–6591.**

The TLR and IL-1R signaling pathways are central in the innate immune response to infection (1). These pathways are activated by microbial structures (e.g., LPS) and cytokines (e.g., IL-1) that use distinct receptors but share common adapter proteins and kinases. TLR and IL-1R signaling results in activation of transcription factors that stimulate cytokine and costimulatory molecule expression (2–4). Although the IL-1R and TLR4 exhibit structurally distinct extracellular domains (2, 5), they share an intracellular Toll-IL-1R resistance (TIR)[3] domain essential for signal transduction (1–5). LPS or IL-1 stimulation triggers receptor aggregation that facilitates recruitment of MyD88 to receptor complexes via interactions of the C-terminal TIR domains within these receptors and MyD88 (1–4). MyD88 also interacts with IL-1R-associated kinase-4 (IRAK-4), via the region of MyD88 situated between its C-terminal TIR and N-terminal death domains (6), and with IRAK-1 through its death domain (7), triggering phosphorylation reactions by IRAK-4 and IRAK-1 (8–10). Once hyperphosphorylated, IRAK-1 releases from the receptor complex and interacts with the TNFR-associated factor 6 complex, leading to activation of MAPK and transcription factors (11–17).

IRAK-4 is essential for mediating cellular activation in response to LPS and IL-1 (10, 18–20). The importance of IRAK-4 is evidenced by the marked susceptibility of IRAK-4-null mice (18) and patients with mutations in *IRAK-4* (19, 20) to bacterial infection that has been correlated with decreased responsiveness to IL-1 and LPS. A patient whom we studied carried two mutations on different alleles of *IRAK-4*: a point mutation (C877T in mRNA) and a 2-nt deletion (620–621del in mRNA), that preclude normal responsiveness to LPS, IL-1, and IL-18 (20). Transfection of HEK293T cells with expression vectors that encode either of the IRAK-4 mutations identified in our patient resulted in the expression of IRAK-4 mutant species with intact death domains, but truncated kinase domains. Upon overexpression, neither mutant IRAK-4 activated endogenous IRAK-1 and both inhibited IL-1-induced IRAK-1 activation, indicating that these mutations render the protein nonfunctional with respect to IRAK-1 activation and confer a dominant-negative effect on normal IRAK-4 (20).

In this study, we used these two truncated IRAK-4 variants as tools to understand more clearly the mechanism(s) by which

*Department of Microbiology and Immunology, University of Maryland, Baltimore, MD 21201; †Clinical Services Program, Science Applications International Corporation-Frederick, Inc., National Cancer Institute-Frederick, Frederick, MD 21702; ‡Laboratory of Host Defense, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892; and §Department of Immunology, Cleveland Clinic Foundation, Cleveland, OH 44195

Received for publication November 8, 2004. Accepted for publication April 7, 2005.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This work was supported by National Institutes of Health Grants AI-18797, AI-44936 (to S.N.V.) AI-059524 (to A.E.M.), and GM-600020 (to X.L.), and National Cancer Institute, National Institutes of Health, under Contract No. NO1-CO-12400.

[2] Address correspondence and reprint requests to Dr. Stefanie N. Vogel, Department of Microbiology and Immunology, University of Maryland, Baltimore, 655 West Baltimore Street, 13-009, Baltimore, MD 21201. E-mail address: svogel@som.umaryland.edu

[3] Abbreviations used in this paper: TIR, Toll-IL-1R resistance; IRAK, IL-1R-associated kinase; WT, wild type.

0022-1767/05/$02.00

they might alter assembly of signaling complexes among IL-1RI, MyD88, and IRAK-1. When overexpressed in the HEK293/IL-1RI cells, truncated IRAK-4 variants exhibited a significantly lower capacity to form complexes with the IL-1RI and endogenous IRAK-1 in response to IL-1 stimulation, and limited recruitment of endogenous IRAK-1 and MyD88 to the IL-1RI. Mutant IRAK-4 proteins associated with endogenous MyD88 constitutively, which was not increased by IL-1, in contrast to the strong IL-1-inducible interaction of wild-type (WT) IRAK-4 with MyD88. Our findings imply that suppressed IL-1-induced signaling is due to sequestration of MyD88 in the cytoplasm by mutant IRAK-4. These findings provide a rationale for development of small molecule inhibitors that mimic these IRAK-4 mutants for mitigation of overexuberant inflammatory responses.

Materials and Methods

Cloning and sequencing of IRAK-4

Genomic DNA or RNA was isolated from healthy volunteers, the patient, or her family, and subjected to PCR and sequencing as described (20).

Reagents and cell culture

PBMC were cultured and stimulated with *Escherichia coli* LPS, and TNF-α mRNA and protein were measured as described (20). Human rIL-1α was obtained from the Biological Resources Branch Preclinical Repository (National Cancer Institute-Frederick, National Institutes of Health). Abs to human IL-1R type I and MyD88 were purchased from Santa Cruz Biotechnology, anti-GFP from Molecular Probes, anti-AU1 from Covance (Covance-Babco), and anti-Flag Ab, M2, from Sigma-Aldrich. Human HEK293T cells (20) and an HEK293 cell line that stably overexpresses IL-1RI (C6 cells; HEK293/IL-1R; Ref. 21) have been described.

Plasmids and transient transfection

pRK7-Flag-IRAK-4 expression vectors encoding WT, C877T (M1), or 620–621del (M2) IRAK-4 variants have been described (20). The pCDNA3-AU-1-MyD88 vector encoding AU1-tagged human MyD88 was provided by Dr. K. Fitzgerald (Massachusetts Medical School, Worcester, MA). Transfections of HEK293T cells were conducted as described (20), using pRK7-Flag-IRAK-4 and pCDNA3-AU1-MyD88 vectors (5 μg each). For transfection of the C6 cell line, cells were cultured overnight in 150-mm TC dishes (5×10^6 cells/dish) and transfected for 3 h with WT or mutant IRAK-4 expression vectors (25 μg/dish) using Superfect Transfection reagent (Qiagen). After 48 h, cell extracts were prepared as described (20).

Coimmunoprecipitation and Western analysis

Cell extracts (500–1000 μg of protein) were incubated overnight with 1 μg of Ab in lysis buffer (20 mM HEPES (pH 7.4), 0.5% Triton X-100, 150 mM NaCl, 12.5 mM β-glycerophosphate, 50 mM NaF, 1 mM DTT, 1 mM sodium orthovanadate, 2 mM EDTA, 1 mM PMSF, and protease inhibitor mixture (Roche)). Thereafter, protein G-agarose beads (Roche; prewashed and resuspended in lysis buffer at 1:1 ratio) were added (40 μl/sample) and incubated for 4 h. Beads were washed four times with lysis buffer, and proteins were separated by SDS-PAGE on 4–20% minigels (Invitrogen Life Technologies) followed by Western analysis (20). Densitometric analysis was conducted using an Alpha Imager 2200 Documentation and Analysis System (Alpha Innotech) to obtain integrated density values.

Results and Discussion

We previously described a patient who suffered from recurrent bacterial infections and exhibited greatly diminished responses to LPS in vivo or to LPS and IL-1 in vitro (22). We determined that this was attributable to distinct mutations on each of the patient's *IRAK-4* alleles: a point mutation, C877T ("M1"), and a 2-nt deletion, 620–621del ("M2") (20). That this was inherited in a Mendelian fashion was first supported by the fact that the patient's father was heterozygous for the point mutation (C877T), whereas her mother and sibling were heterozygous for the deletion mutation (20). Further analysis of the grandparents extends our findings (Fig. 1*A*): the maternal grandfather was a heterozygous carrier of the 620–621del mutation, the paternal grandmother carried both WT and the C877T alleles of *IRAK-4*, whereas the maternal grandmother and paternal grandfather were homozygous for WT *IRAK-4*. Consistent with our earlier findings (20), heterozygous expression of either IRAK-4 mutation was not associated with recurrent infection in the carrier grandparents and did not preclude normal induction of LPS-inducible TNF-α mRNA (Fig. 1*B*) or protein (*C*)

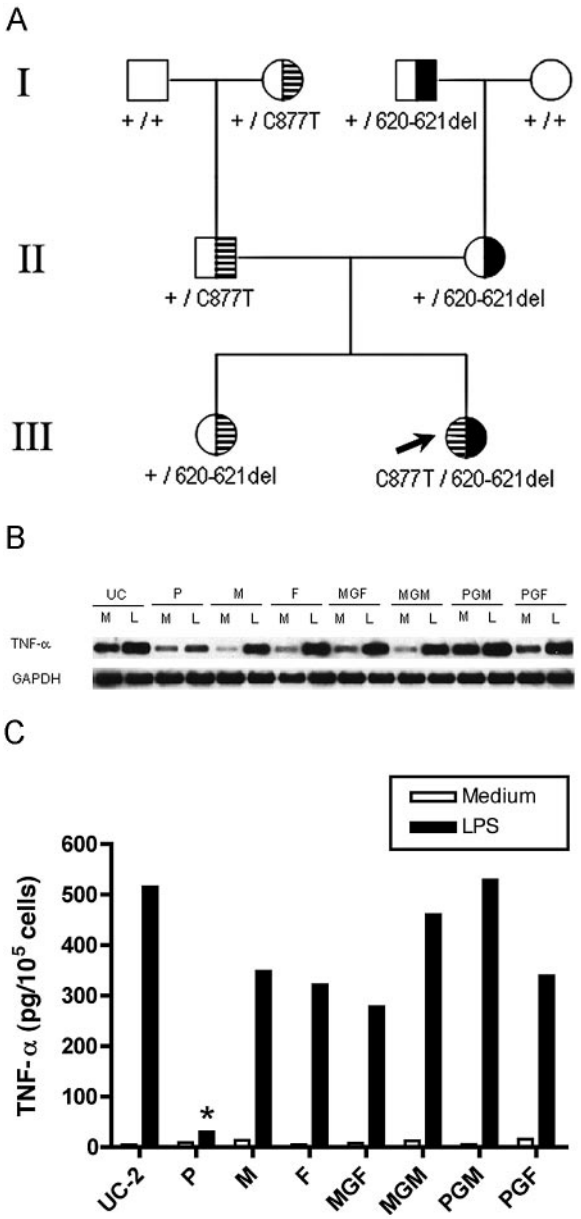

FIGURE 1. Inheritance of C877T and 620–621del IRAK-4 mutations in the patient's family. *A*, C877T (hatched) and 620–621del (black) IRAK-4 mutations were identified by sequence analysis of genomic DNA. Males are shown as squares, and females as circles. I, Grandparents; II, parents; III, patient and sibling. *B*, PBMC from unrelated control (UC), patient (P), mother (M), father (F), maternal grandfather (MGF) and grandmother (MGM), and paternal grandfather (PGF) and grandmother (PGM) were treated with medium or 100 ng/ml LPS for 4 h. RNA was isolated and subjected to RT-PCR to measure TNF-α mRNA relative to GAPDH mRNA levels. *C*, TNF-α secretion in supernatants from PMBC stimulated with medium or LPS.

in PBMC obtained from the family members, in contrast to the patient's minimal response to LPS.

In contrast to the normal homozygous expression of WT IRAK-4, overexpression of the truncated forms of IRAK-4 in HEK293T cells blocked IL-1-induced, IRAK-4-dependent activation of endogenous IRAK-1 kinase activity (20), and the point mutation (M1) was found to be more strongly inhibitory than the deletion mutation (M2). This implies that expression levels of mutated IRAK-4 proteins by the patient's heterozygotic parents, grandparents, and sister are insufficient to block activity of their WT IRAK-4, in contrast to a predominance of mutant IRAK-4 variants over endogenous normal IRAK-4 achieved under conditions of overexpression. The failure of our patient's PBMC to respond to LPS to increase TNF-α mRNA or protein (Fig. 1, *B* and *C*; Ref. 20) and GM-CSF (20) or to activate MAPKs and transcription factors (20) support the conclusion that at least one WT copy of *IRAK-4* is necessary for mounting a normal response.

IL-1 stimulation results in recruitment of MyD88, IRAK-4, and IRAK-1 to the IL-1R (3, 7–10). Interaction of IRAK-4 with IRAK-1 induces IRAK-1 phosphorylation and activation, triggering downstream signaling cascades (23). To dissect the molecular mechanisms by which truncations within the IRAK-4 kinase domain diminish signaling, we hypothesized that, upon overexpression, mutant IRAK-4 molecules cause aberrant IL-1-induced interactions with signaling components of the IL-1R complex. To test this hypothesis, we examined IL-1-induced recruitment of WT and mutant IRAK-4 species to the IL-1R in HEK293 cells stably transfected with IL-1RI (to increase the sensitivity of detection). Following transient transfection with Flag-tagged WT or mutant IRAK-4, cells were stimulated with medium or rIL-1α. WT, M1, and M2 IRAK-4 proteins were immunoprecipitated with anti-Flag Ab, and immunoprecipitates were immunoblotted with anti-IL-1RI Ab to compare the capacity of the IRAK-4 species to interact with IL-1RI. Fig. 2, *panel 2*, shows that IL-1α stimulation results in differential association of WT, M1, and M2 IRAK-4 species with IL-1RI, under conditions where levels of the IL-1RI (*panel 1*) and WT, M1, and M2 IRAK-4 (*panel 3*) were comparably expressed. Although WT IRAK-4 exhibited the strongest capacity to form complexes with IL-1RI in response to IL-1α stimulation, M2 associated less strongly, and recruitment of M1 was barely detectable, consistent with the relative capacity of M1 vs M2 to block signaling (20). Quantitative densitometric analysis confirmed the weak IL-1-inducible interaction of the IL-1RI with M1 (15.8%) and intermediate interaction with M2 (47.1%) compared with WT IRAK-4 (100%). These data were confirmed by analyzing IL-1R immunoprecipitates by immunoblotting with anti-Flag Ab for the presence of IRAK-4 species in the complexes (data not shown). Collectively, these results indicate that mutations in the IRAK-4 kinase domain inhibit its association with the IL-1R upon stimulation with IL-1α.

Because IRAK-4 has been reported to facilitate recruitment of IRAK-1 to the IL-1R (23), we next hypothesized that the diminished IL-1-mediated association of truncated IRAK-4 with the IL-1R may also result in impaired recruitment of endogenous IRAK-1. Fig. 2, *panel 4*, demonstrates that IL-1-induced recruitment of endogenous IRAK-1 to the IL-1R complex was also significantly inhibited in cells that overexpress mutant IRAK-4 variants. Although much less activated IRAK-1 was found in association with the IL-1RI than in whole-cell lysates (Fig. 2, *panel 3* vs *4*), M1 and M2 were significantly less capable than WT IRAK-4 of mediating this recruitment. Densitometric analysis of these blots confirmed these findings (M1, 33.2%; M2, 29.5%). Therefore, truncations in the IRAK-4 kinase domain impair its ability to recruit and activate IRAK-1 at the IL-1R in response to IL-1α.

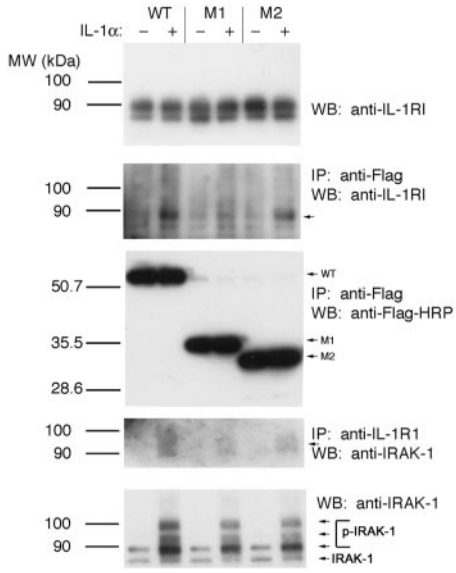

FIGURE 2. IRAK-4 proteins with truncated kinase domains exhibit suppressed IL-1-inducible recruitment to the IL-1R and inhibit IL-1-inducible recruitment of endogenous IRAK-1 to the IL-1RI. Flag-tagged WT, M1, or M2 IRAK-4 were overexpressed in HEK293/IL-1R cells followed by treatment for 15 min with medium or 100 ng/ml rIL-1α. The *top panel* shows total expression of IL-1R detected by Western analysis of cell lysates with anti-IL-1R Ab. Cell lysates were immunoprecipitated with anti-Flag Ab and immune complexes subjected to Western analysis with both anti-IL-1R Ab (*second panel*, detection of IL-1R association with IRAK-4) or anti-Flag Ab (*third panel*, total expression of transfected IRAK-4 species). In addition, IL-1RI was immunoprecipitated from cell lysates with anti-IL-1RI Ab and subjected to immunoblot analysis with anti-IRAK-1 Ab (*panel 4*, detection of IRAK-1 in association with IL-1R). The *bottom panel* shows total IRAK-1 protein detected in cell lysates before immunoprecipitation. Results are from a representative experiment ($n = 3$).

We next examined whether truncations in the IRAK-4 kinase domain alter interactions of IRAK-4 with endogenous IRAK-1 upon IL-1α stimulation. WT and mutant IRAK-4 species were again overexpressed in HEK293/IL-1R cells, and IL-1α -inducible associations of endogenous IRAK-1 with Flag-tagged transfected IRAK-4 variants were measured. Fig. 3, *panel 2*, shows that IL-1α triggered strong association of WT IRAK-4 with activated IRAK-1, as evidenced by the presence of high molecular mass IRAK-1 species (>80 kDa). In contrast, significantly less activated IRAK-1 was found in complex with M1 IRAK-4. Densitometric analysis confirmed that IL-1α stimulation led to only a 24.5% association of M1 with endogenous IRAK-1 when compared with the interaction of WT IRAK-4 with IRAK-1 (100%). Similar results were observed for M2 (data not shown). These results confirm that the IL-1-inducible association of IRAK-4 with IRAK-1, and activation of IRAK-1, is greatly diminished as a consequence of truncations within the IRAK-4 kinase domain.

Finally, we examined the capacity of WT and mutant IRAK-4 species to interact with MyD88. It had been reported previously that overexpression of a "kinase dead" IRAK-4 (IRAK-4 KK213AA, obtained by site-directed mutagenesis of

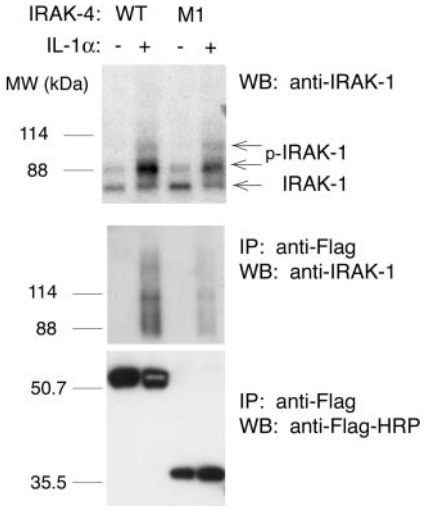

FIGURE 3. Suppressed IL-1α-induced association of activated IRAK-1 with M1 IRAK-4. WT or M1 Flag-tagged IRAK-4 variants were overexpressed in HEK293/IL-1R cells followed by stimulation for 15 min with medium or 100 ng/ml rIL-1α. IRAK-4 proteins were immunoprecipitated with anti-Flag Ab, and IRAK-4 immune complexes were examined with anti-IRAK-1 Ab or anti-Flag-HRP Ab by Western analysis. The *top panel* shows total IRAK-1 protein expression analyzed in cell lysates before immunoprecipitation. Results are from a representative experiment ($n = 5$).

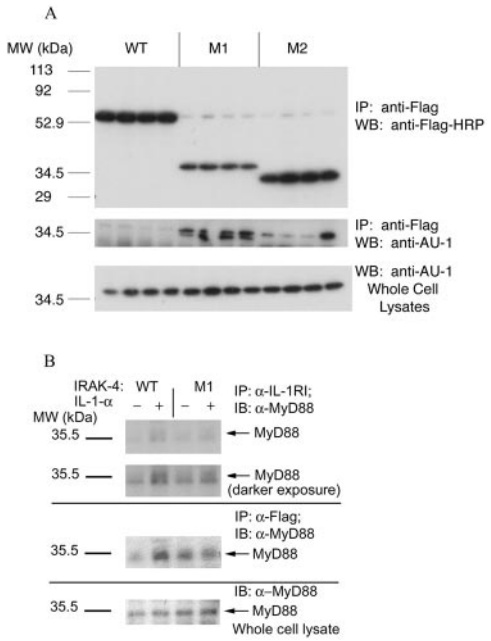

FIGURE 4. Constitutive and IL-1-inducible interactions of MyD88 with WT, M1, and M2 forms of IRAK-4. *A*, HEK293T cells were transiently cotransfected with pCDNA3-AU-1-huMyD88 and either WT or mutant Flag-tagged forms of IRAK-4 (quadruplicates for each transfection). Anti-Flag immunoprecipitates and whole-cell lysates were subjected to Western analysis to detect immunoprecipitated IRAK-4 (*top panel*), AU-1-MyD88 in association with IRAK-4 (*center panel*), and total AU-1-MyD88 in whole-cell lysates. *B*, WT and M1 IRAK-4 were overexpressed in HEK293/IL-1R cells, followed by treatment with medium or 100 ng/ml rIL-1α for 15 min. Cell lysates were divided and subjected to immunoprecipitation with either anti-IL-1RI Ab (*top panel*), or anti-Flag Ab (*middle panel*). IL-1RI (*top*) and IRAK-4 (*middle*) immune complexes were immunoblotted with anti-MyD88 Ab to detect endogenous MyD88. The *bottom panel* shows total expression of endogenous MyD88 in cellular extracts before immunoprecipitation. Results are from a representative experiment ($n = 3$).

two lysine residues in the ATP binding pocket to alanine residues) and MyD88 resulted in their constitutive association (10). Therefore, we sought to determine whether truncations in the kinase domain of IRAK-4 associated with hyporesponsiveness to LPS and IL-1 (20) would affect its ability to interact with MyD88. To this end, the HEK293T cells were transiently transfected with an AU-1-epitope-tagged MyD88 expression vector and expression vectors that encode either WT, M1, or M2 forms of *IRAK-4*, and the interaction of IRAK-4 with MyD88 was analyzed. Fig. 4*A* demonstrates that M1 and M2 mutant variants of IRAK-4 interact with MyD88 significantly more strongly than WT IRAK-4, with M1 being greater than M2. These results were confirmed when WT and mutant IRAK-4 species were overexpressed in HEK293 cells stably transfected with the YFP-tagged MyD88 expression vector (kindly provided by Dr. K. Fitzgerald) and in HEK293/IL-1R cells (data not shown). These data indicate that truncated, kinase-inactive IRAK-4 proteins may preferentially bind to and sequester MyD88, preventing its interaction with kinase-competent IRAK-4 molecules upon IL-1 stimulation, thereby disrupting downstream signaling. In support of this conclusion, IL-1-mediated recruitment of endogenous MyD88 to the IL-1R was much greater in the presence of WT IRAK-4 than in the presence of the truncated IRAK-4 proteins. Whereas M1 IRAK-4 also associated constitutively with endogenous MyD88, no constitutive association of endogenous MyD88 with WT IRAK-4 was detected in medium-treated cells (Fig. 4*B*, *panel 1*). Although IL-1α stimulation resulted in a strong interaction between endogenous MyD88 and WT IRAK-4, M1 IRAK-4 association with endogenous MyD88 in response to IL-1α remained unchanged and was significantly lower than IL-1-inducible WT IRAK-4-MyD88 association (Fig. 4*B*, *panel 2*).

In conclusion, this study demonstrates several mechanisms by which overexpression of truncated, kinase-deficient forms of IRAK-4 may disrupt signaling induced by LPS or IL-1. First, mutant IRAK-4 variants are recruited to the IL-1RI to a much lesser extent than WT IRAK-4. Recruitment of IRAK-4 to the IL-1R is independent of IRAK-1 and MyD88 because it occurs in both IRAK-1- and MyD88-null cells (23). Therefore, our findings suggest that the kinase domain of IRAK-4 is required for its IL-1-inducible association with the IL-1R. In addition, because IRAK-4 facilitates recruitment of IRAK-1 to the IL-1R (23), our observation of decreased IRAK-1 association with the IL-1RI and IRAK-4, as well as its poor activation in cells that express kinase-defective IRAK-4 variants, suggests that the mutations preclude the proper interaction among IL-1RI, IRAK-4, and IRAK-1. Lastly, M1 and M2 IRAK-4 molecules interact constitutively with MyD88 more strongly and decrease IL-1-inducible recruitment of MyD88 to the IL-1R. Coupled with our finding of suppressed mutant IRAK-4 and MyD88 recruitment to the IL-1R upon IL-1α stimulation, this suggests that overexpressed mutant IRAK-4 species have the capacity to sequester MyD88 in the cytoplasm and limit its recruitment to the IL-1R and association with other components of the IL-1R complex. Hence, sequestration of MyD88 in the cytoplasm by mutant IRAK-4 proteins would be predicted to suppress MyD88-mediated recruitment of IRAK-1 to the IL-1R in response to IL-1, where IRAK-1 undergoes phosphorylation by IRAK-4. In this respect, it is noteworthy that truncations in the

kinase domain of IRAK-4 markedly impair its capacity to interact with IRAK-1 in response to IL-1 stimulation, as shown in the present study. Collectively, these data indicate that, when IRAK-4 proteins with truncations in the kinase domain are overexpressed, they can suppress IL-1-inducible recruitment of WT IRAK-4 to the IL-1RI and its association with IRAK-1, while enabling sequestration of MyD88. Using Western analysis, Picard et al. (19) failed to detect IRAK-4 protein expression in cell lines derived from homozygous recessive patients with the M1 IRAK-4 mutation. Thus, in such patients, the failure to signal is most likely due to a lack of WT IRAK-4, rather than any disruption in receptor complex formation as is seen when mutant IRAK-4 variants are overexpressed in HEK293 cells. Nonetheless, the finding that overexpressed, truncated IRAK-4 variants block normal IL-1-induced signaling by disrupting formation of the receptor complex provides a potential therapeutic approach for the development of small mimetics of truncated IRAK-4 proteins that could possibly mitigate hyperinflammatory diseases by sequestering MyD88 in the cytoplasm, thus precluding activation of IRAKs and subsequent downstream signaling.

Disclosures

The authors have no financial conflict of interest.

References

1. Takeda, K., T. Kaisho, and S. Akira. 2003. Toll-like receptors. *Annu. Rev. Immunol.* 21: 335–376.
2. Medzhitov, R. 2001. Toll-like receptors and innate immunity. *Nat. Rev. Immunol.* 1: 135–145.
3. Vogel, S. N., K. A. Fitzgerald, and M. J. Fenton. 2003. TLRs: differential adapter utilization by Toll-like receptors mediates TLR-specific patterns of gene expression. *Mol. Interv.* 3: 466–477.
4. Means, T. K., D. T. Golenbock, and M. J. Fenton. 2000. Structure and function of Toll-like receptor proteins. *Life Sci.* 68: 241–258.
5. Mitcham, J. L., P. Parnet, T. P. Bonnert, K. E. Garka, M. J. Gerhart, J. L. Slack, M. A. Gayle, S. K. Dower, and J. E. Sims. 1996. T1/ST2 signaling establishes it as a member of an expanding interleukin-1 receptor family. *J. Biol. Chem.* 271: 5777–5783.
6. Burns, K., S. Janssens, B. Brissoni, N. Olivos, R. Bayaert, and J. Tschopp. 2003. Inhibition of interleukin-1 receptor signaling through the alternatively spliced, short form of MyD88 is due to its failure to recruit IRAK-4. *J. Exp. Med.* 197: 263–268.
7. Wesche, H., W. J. Henzel, W. Shillinglaw, S. Li, and Z. Cao. 1997. MyD88: an adapter that recruits IRAK to the IL-1 receptor complex. *Immunity* 7: 837–847.
8. Burns, K., F. Martinon, C. Esslinger, H. Pahl, P. Schneider, J.-L. Bodmer, F. Di Marco, L. French, and J. Tschopp. 1998. MyD88, an adaptor protein involved in IL-1 signaling. *J. Biol. Chem.* 273: 12203–12209.
9. Muzio, M., J. Ni, P. Feng, and V. Dixit. 1997. IRAK (Pelle) family member IRAK-2 and MyD88 as proximal mediators of IL-1 signaling. *Science* 278: 1612–1615.
10. Li, S., A. Strelow, E. J. Fomtana, and H. Wesche. 2002. IRAK-4: a novel member of the IRAK family with the properties of an IRAK-kinase. *Proc. Natl. Acad. Sci. USA* 99: 5567–5572.
11. Cao, Z., J. Xiong, M. Takeuchi, T. Kurama, and D. V. Goeddel. 1996. TRAF6 is a signal transducer for interleukin-1. *Nature* 383: 443–446.
12. Yamin, T.-T., and D. K. Miller. 1997. The interleukin-1 receptor-associated kinase is degraded by proteasomes following its phosphorylation. *J. Biol. Chem.* 272: 21540–21547.
13. Zhang, F. X., C. J. Kirschning, R. Mancinelli, X. P. Xu, Y. Lin, E. Faure, A. Mantovani, M. Rothe, M. Muzio, and M. Arditi. 1999. Bacterial lipopolysaccharide activates nuclear factor-κB through interleukin-1 signaling mediators in cultured human dermal endothelial cells and mononuclear phagocytes. *J. Biol. Chem.* 274: 7611–7614.
14. Irie, T., T. Muta, and K. Takeshige. 2000. TAK1 mediates an activation signal from Toll-like receptor(s) to nuclear factor-κB in lipopolysaccharide-stimulated macrophages. *FEBS Lett.* 467: 160–164.
15. Takaesu, G., S. Kishida, A. Hiyama, K. Yamaguchi, H. Shibuya, K. Irie, J. Ninomiya-Tsuji, and K. Matsumoto. 2000. TAB2, a novel adaptor protein, mediates activation of TAK1 MAPKKK by linking TAK1 to TRAF6 in the IL-1 signal transduction pathway. *Mol. Cell* 5: 649–658.
16. Takaesu, G., J. Ninomiya-Tsuji, S. Kishida, X. Li, G. Stark, and M. Matsumoto. 2001. Interleukin-1 (IL-1) receptor-associated kinase leads to activation of tak 1 by inducing tab2 translocation in the IL-1 signaling pathway. *Mol. Cell. Biol.* 21: 2475–2484.
17. Kopp, E., R. Medzhitov, J. Carothers, C. Xiao, I. Douglas, C. A. Janeway, and S. Ghosh. 1999. ECSIT is an evolutionary conserved intermediate in the Toll/IL-1 signal transduction pathway. *Genes Dev.* 13: 2059–2071.
18. Suzuki, N., S. Suzuki, G. S. Duncan, D. G. Millar, T. Wada, C. Mirtsos, H. Takada, A. Wakeham, A. Itie, S. Li, et al. 2002. Severe impairment of interleukin-1 and Toll-like receptor signalling in mice lacking IRAK-4. *Nature* 416: 750–756.
19. Picard, C., A. Puel, M. Bonnet, C. L. Ku, J. Bustamante, K. Yang, C. Soudais, S. Dupuis, J. Feinberg, C. Fieschi Elbim, et al. 2003. Pyogenic bacterial infections in humans with IRAK-4 deficiency. *Science* 299: 2076–2079.
20. Medvedev, A. E., A. Lentschat, D. B. Kuhns, J. C. Blanco, C. Salkowski, S. Zhang, M. Arditi, J. I. Gallin, and S. N. Vogel. 2003. Distinct mutations in IRAK-4 confer hyporesponsiveness to lipopolysaccharide and interleukin-1 in a patient with recurrent bacterial infections. *J. Exp. Med.* 198: 521–531.
21. Li, X., M. Commane, Z. Jiang, and G. R. Stark. 2001. IL-1-induced NFκB and c-Jun N-terminal kinase (JNK) activation diverge at IL-1 receptor-associated kinase (IRAK). *Proc. Natl. Acad. Sci. USA* 98: 4461–4465.
22. Kuhns, D. B., D. A. Long-Priel, and J. I. Gallin. 1997. Endotoxin and IL-1 hyporesponsiveness in a patient with recurrent bacterial infections. *J. Immunol.* 158: 3959–3964.
23. Qin, J., Z. Jiang, Y. Qian, J. L. Casanova, and X. Li. 2004. IRAK4 kinase activity is redundant for interleukin-1 (IL-1) receptor-associated kinase phosphorylation and IL-1 responsiveness. *J. Biol. Chem.* 279: 26748–26753.