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Distinct Control of MyD88 Adapter-dependent and Akt Kinase-regulated Responses by the Interleukin (IL)-1RI Co-receptor, TILRR*[S]

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Xiao Zhang[‡], Gemma Montagut Pino[‡], Freya Shephard[‡], Endre Kiss-Toth[§], and Eva E. Qwarnstrom[‡¶1]

From the [‡]Cell Biology Group and [§]Cell Signalling Group, Department of Cardiovascular Science, Medical School, University of Sheffield, Sheffield S10 2RX, United Kingdom and the [¶]Department of Pathology, University of Washington, Seattle, Washington 98195-7470

Background: The inflammatory regulator, IL-1RI (interleukin-1 type-1 receptor), is controlled through its co-receptor TILRR (Toll-like and IL-1 receptor regulator).
Results: Amplification of inflammatory responses and cell survival through this receptor system depends on distinct residues within the TILRR core protein.
Conclusion: TILRR has the capacity to selectively regulate inflammatory responses and cell survival.
Significance: Information on signal specificity is central to understanding response control.

Inflammatory responses are controlled through members of the interleukin-1 receptor (IL-1R)/Toll-like receptor superfamily. Our earlier work demonstrates that the IL-1 receptor type 1 (IL-1RI) co-receptor, Toll-like and IL-1 receptor regulator (TILRR), amplifies IL-1 activation of NF-κB and inflammatory genes. Here we show that TILRR similarly promotes IL-1-induced anti-apoptotic signals and reduces caspase-3 activity. Further, the TILRR-induced effects on cell survival and inflammatory responses are controlled through distinct parts of the IL-1RI regulatory Toll IL-1 receptor (TIR) domain. Alanine-scanning mutagenesis identified a functional TILRR mutant (R425A), which blocked increases in cell survival and upstream activation of Akt but had no effect on amplification of MyD88-dependent inflammatory responses. A second mutant (D448A) blocked TILRR potentiation of MyD88-dependent signals and inflammatory activation but had no impact on cell survival. Secondary structure predictions suggested that the mutations induce distinct alterations in the α-helical structure of the TILRR core protein. The results indicate a role for TILRR in selective amplification of NF-κB responses through IL-1RI and suggest that the specificity is determined by changes in receptor conformation and adapter protein recruitment.

Toll-like and IL-1 receptors control inflammatory responses and cell survival (1–4). The processes involve activation of the transcription factor NF-κB and induction of inflammatory and anti-apoptotic genes (1, 5, 6).

Signal amplification of these systems originates from the TIR[2] domain, the evolutionarily conserved stretch of 200 amino acids common to all members of the Toll-like and IL-1 receptor family (2, 7). Activation of inflammatory responses requires recruitment of the MyD88 adapter to the receptor complex, whereas cell survival signals are MyD88-independent and controlled by the p85 subunit of PI3-kinase and the protein kinase Akt (8).

We have identified a novel IL-1RI co-receptor, Toll-like and IL-1 receptor regulator (TILRR), a spliced variant of FREM1 of the FRAS1 family (9), which is independently regulated through a 5′-UTR and an initiator codon, within a unique exon. Our earlier studies show that TILRR associates with IL-1RI to amplify IL-1-induced activation of NF-κB and inflammatory genes (10). Here we demonstrate that TILRR similarly potentiates IL-1-induced anti-apoptotic responses. Further, amplification of inflammatory and survival signals is controlled through discrete functional residues within the TILRR core protein, predicted to impact its secondary structure.

EXPERIMENTAL PROCEDURES

Cell Culture—HeLa Cells were grown in DMEM (Invitrogen), detached using EDTA (5 mM, Sigma), and plated as described previously (10). Cells were transfected 24 h after plating and incubated with IL-1β (1 nM) 24 h after transfection. Apoptosis was induced by incubating with TNF-α (Promega, 10 ng/ml) and cycloheximide (Sigma, 10 μg/ml), for various times, starting 40 h after transfection. Conditions for various assays, including activation times and cDNA levels, and optimization of all transfection protocols were determined based on initial experiments.

Plasmid cDNAs and siRNA Constructs and Transfection—Plasmids containing wild type or mutant TILRR cDNAs, TILRR-specific or random siRNAs, wild type or mutant IL-1R1 cDNAs, dominant negative (DN) MyD88 or DNTRAF6, or the IL-8 promoter (IL-8-Luc, -EGFP) were transfected by calcium phosphate, as described previously (10), or by PolyFect (Qiagen) according to the manufacturer's instructions. All experiments used constant levels of DNA and included empty vector (mock) or random siRNA (no genomic equivalent) as controls, respectively.

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[1] To whom correspondence should be addressed: Cell Biology Group, Dept. of Cardiovascular Science, University of Sheffield, Medical School, Beech Hill Road, Sheffield S10 2RX, United Kingdom. Tel.: 44-114-271-318 or 44-114-271-2313; Fax: 44-114-271-1863; E-mail: e.qwarnstrom@sheffield.ac.uk.

[2] The abbreviations used are: TIR, Toll/Interleukin-1 receptor; TILRR, Toll-like and IL-1 receptor regulator; Akt, protein kinase B (PKB); MyD88, myeloid differentiation primary response gene (88); IL-1RI, interleukin-1 receptor type I; DN, dominant negative; EGFP, enhanced green fluorescent protein; DEVD-AFC, DEVD-7-amino-4-trifluoromethyl coumarin.

Mutagenesis—The TILRR core protein was subjected to alanine-scanning mutagenesis, and sites were selected based on predicted impact on secondary protein structure (10). IL-1RI-TIR alanine-scanning mutants were identified by sequence alignment as described (11). Optimal transfection levels were assessed for each mutant by using a range of cDNA concentrations, and cell surface expression levels were determined by FACS analysis.

FACS Analysis—Cells transfected with empty vector (mock) or cDNAs containing wild type or mutant TILRR were fixed (2% paraformaldehyde, 15 min, 4 °C) and incubated with a custom anti-TILRR rabbit-polyclonal antibody (2.5 μg/ml, 30 min 4 °C, Eurogentec) or with a normal rabbit IgG (specificity control) and with a FITC-conjugated anti-rabbit antibody (2 μg/ml) using conventional methods, as described previously (10), and levels of cell surface expression of wild type TILRR and TILRR mutants were determined by flow cytometry.

Single Cell Analysis—Cells were transfected with IL-8-EGFP, and levels of the fusion protein were monitored continuously by light microscopy. Fluorescence levels were quantitated using NIH Image, as described previously (12).

Luciferase Assay—Luciferase activity was determined using a Dual-Luciferase reporter assay system (Promega) and conventional procedures, as described previously (10).

Protein Extraction and Quantitation—Cells were resuspended in extraction buffer and supernatant diluted in sample buffer or resuspended in loading buffer and denatured (95 °C, 5 min), as described previously (10). Total protein was quantitated by the BCA assay (Pierce).

TUNEL Staining—The ApoBrdU DNA fragment kit (BioVision) was used according to the manufacturer's instructions. EDTA (5 mM)-detached cells were washed in PBS, fixed (2% paraformaldehyde, 15 min, 4 °C), and permeabilized (EtOH 70%, 4 °C). Cells were incubated in terminal deoxynucleotidyl transferase reaction buffer (2.0 μl of BrdUTP stock solution, 15 units of terminal deoxynucleotidyl transferase, 5 μl of 10 mM $CoCl_2$, 33.5 μl of H_2O; 60 min 37 °C) with an anti-BrdU-FITC antibody (1/20 in resin, 1 h), washed, and immersed in PI/RNase staining solution (30 min, room temperature). Cultures were analyzed using a FITC/PI fluorescence sorter (BD Biosciences) with appropriate voltage and flow determined by levels in control samples.

Caspase-3 Activity—This was measured using a fluorometric assay kit (BioVision) according to manufacturer's instructions. Cells were extracted in lysis buffer (50 μl, 10 min,) and incubated in buffer (BioVision) containing caspase-3 substrate Ac-DEVD-AFC (BioVision) at 37 °C, and fluorescence was measured every 15–30 min for 2 h, using a fusion plate reader with 400-nm excitation and 505-nm emission.

Cross-linking and Immunoprecipitation—Cells were incubated with amine-directed cross-linker, disuccinimidyl suberate (DSS) (membrane-permeable, 5 mM, 30 min, room temperature, Pierce), and samples were extracted as above. Equal amounts were immunoprecipitated using an anti-IL-1RI antibody (2 μg/ml, Santa Cruz Biotechnology) and protein A/G+ agarose beads (20 μl/ml, 4 °C/ON).

Western Analysis—Samples were resolved on SDS-PAGE (4, 6, 8, or 4–12%) and transferred to PVDF. Membranes were incubated with a rabbit polyclonal anti-TILRR (1:1000, custom Eurogentec), anti-IL-1R1 (1:1000, Santa Cruz Biotechnology), anti-MyD88 (1:1000, Santa Cruz Biotechnology), anti-phospho-Akt (1:500, Cell Signaling), or anti-Akt (1:1000, Cell Signaling) or with anti-IgG or anti-β-actin used as loading controls (1:1000, Santa Cruz Biotechnology) or with secondary antibody alone (specificity control) followed by incubation with an HRP-conjugated secondary antibody (1:5000, Santa Cruz Biotechnology or 1:2000 Cell Signaling) and visualized by ECL (all Santa Cruz Biotechnology). Band intensity was determined using two exposures for each blot and quantitated by NIH Image or ImageJ (version 1.66f or J64), as described previously (10).

Secondary Structure Predictions—Secondary structure predictions of the wild type and mutant TILRR proteins were carried out using the Lasergene package (DNASTAR) and the "Garnier-Robson" method, as described (13, 14).

Statistical Analysis—Statistical analysis was carried out by unpaired Student's *t* test, using the GraphPad Prism program.

RESULTS AND DISCUSSION

TILRR Potentiates IL-1-induced Inflammatory Responses and Cell Survival—Single cell analysis using IL-8-EGFP as the readout for inflammatory gene activation, as described under "Experimental Procedures," demonstrated a 2-fold increase in IL-1-induced promoter activity in the presence of TILRR cDNA, whereas TILRR siRNA caused a 40% reduction (Fig. 1*A*).

Parallel experiments showed that TILRR similarly potentiated IL-1-induced cell survival (Fig. 1*B*). Incubation with IL-1 alone reduced TNF and cycloheximide-induced caspase-3 activity by 20 $\pm$ 7%. In the presence of TILRR cDNA, the effect was 60 $\pm$ 5% and similar to levels in cells transfected with the NF-κB subunit relA, used as a positive control. TUNEL staining demonstrated that increasing TILRR expression caused a greater than 40% reduction in cell death (data not shown).

Additional experiments showed that the TILRR-induced decrease in caspase-3 activity was concentration-dependent and comparable with effects by IL-1RI cDNA (Fig. 1*C*, *panels i* and *ii*). Reductions induced by IL-1RI were blocked by TILRR siRNA in a concentration-dependent manner (Fig. 1*C*, *panel iii*), demonstrating the significance of TILRR expression in the IL-1RI-induced inhibition.

TILRR Increases Inflammatory Responses and Cell Survival through Distinct IL-1RI-TIR Domains—Subsequent experiments used a series of alanine-scanning mutants of the IL-1RI-TIR domain, as described previously (11). These showed that substitution of residue Asp-369 (box 1) and Arg-411 (box 2) reduced IL-1-induced activation in agreement with our earlier studies (10, 11) (data not shown). Blocking TILRR expression in the presence of the wild type receptor construct or the box 1 mutants (D369A, Y374F) caused a reduction in caspase-3 activity by 75–80%, whereas substitutions in box 2 (R411A, K428A) rendered the receptor insensitive to TILRR expression (Fig. 1*D*). In contrast, TILRR siRNA reduced inflammatory responses in the presence of the wild type receptor or the box 2

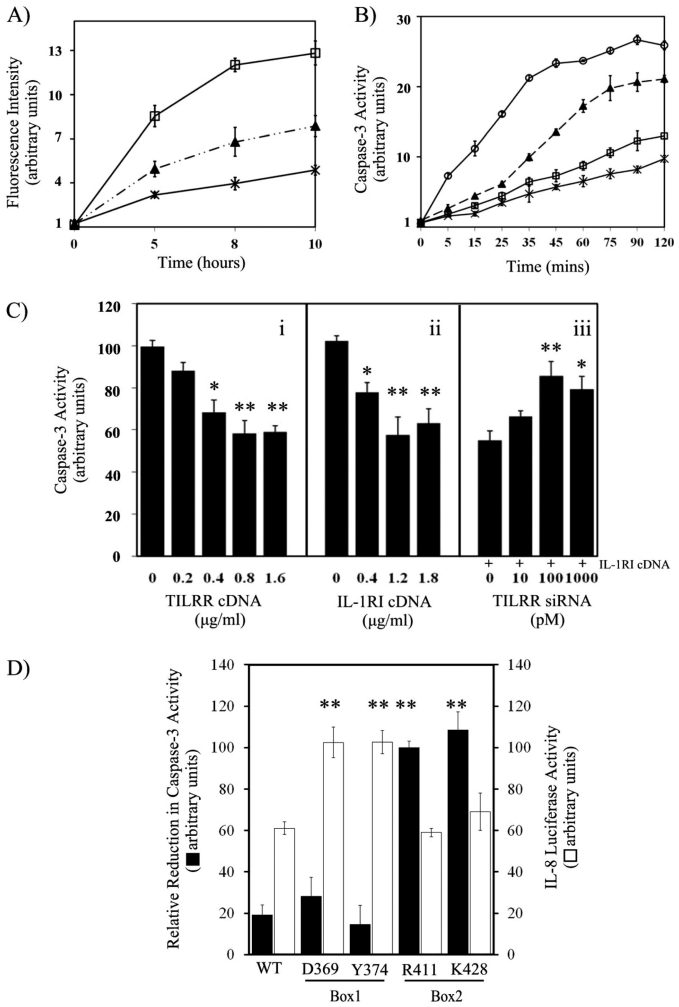

FIGURE 1. **TILRR controls IL-1-induced inflammatory responses and anti-apoptotic survival signals through distinct portions of IL-1RI-TIR-domain.** *A*, HeLa cells were plated in 8-well chamber slides and transfected with IL-8-EGFP (0.2 μg/ml), together with empty vector (mock, ▲, 0.2 μg/ml), TILRR cDNA (□, 0.2 μg/ml), or TILRR siRNA (X, 100 pM), and stimulated with IL-1β (1 nM) for the times indicated. Relative fluorescence intensity was determined by microscopy and quantitated using NIH Image. Random siRNA (100 pM, control not shown) had no impact on activity. Data are expressed relative to values in unstimulated samples for each condition and show mean ± S.E. of three independent experiments. $p < 0.01$ for both TILRR cDNA and siRNA in relation to empty vector control at 10 h. *B*, HeLa cells (10^6) transfected with empty vector (mock, ▲, 0.5 μg/ml), TILRR cDNA (□, 0.5 μg/ml), or RelA cDNA (positive control) (X, 1 μg/ml) were incubated with IL-1β (1 nM, 3 h) and treated with TNF-α (10 ng/ml) and cycloheximide (10 μg/ml) for 3 h. Negative controls (○) were transfected with empty vector and left unstimulated, but similarly treated with TNF-α and cycloheximide. Caspase-3 activity was determined at various times, as indicated. Data are expressed relative to levels in untreated samples and represent mean ± S.E. of three independent experiments. $p < 0.001$ at 120 min, for TILRR cDNA + IL-1 stimulation (□) relative to empty vector control + IL-1 stimulation (▲). *C*, HeLa cells were untransfected or transfected with increasing concentrations of TILRR cDNA (*panel i*) or IL-1RI cDNA (*panel ii*) as indicated or transfected with a constant level of IL-1RI cDNA (1.2 μg/ml) and increasing levels of TILRR siRNA (*panel iii*), as indicated. All cultures were subsequently treated with TNF-α and cycloheximide, and caspase-3 activity was measured, as in *B*, 1 h after treatment. Data are expressed relative to levels in cells transfected with empty vector (*panels i* and *ii*) or random siRNA (*panel iii*). Each panel represents mean ± S.E. of three independent experiments. *, $p < 0.05$, **, $p < 0.01$. *D*, HeLa cells were grown in a 96-well plate and transfected with IL-1RI-TIR domain mutants (1.25 μg/ml) (*box1*, Asp-369, Tyr-374; *box 2*, Arg-411, Lys-428), in the presence of TILRR siRNA (100 pM) or random siRNA (100 pM). (■), cells were stimulated with IL-1β (1 nM, 3 h) followed by treatment with TNF-α and cycloheximide, and caspase-3 activity was measured, as in *B*, 1 h after treatment. (□), cells were co-transfected with pIL-8-Luc (1.25 μg/ml) and TK-RL (0.4 μg/ml) and stimulated with IL-1β (10 nM, 6 h), and luciferase activity was measured. Data show activity in the presence of the TILRR siRNA and are expressed relative to levels in cells transfected with the respective mutant and random siRNA, and represent mean ± S.E. of three independent experiments. **, $p < 0.01$.

mutants, whereas the effect was abrogated by mutations in box 1 (Fig. 1*D*).

These data show that TILRR amplification of both inflammatory responses and cell survival is controlled through the TIR domain of IL-1RI. Further, they suggest that TILRR regulation of MyD88-dependent activation involves the signature sequence of the TIR domain (box 1), whereas its effects on anti-apoptotic signals rely on sequences within the loop structure of box 2 (11, 15).

IL-1-induced Inflammatory Responses and Cell Survival Are Dependent on Select Residues within the TILRR Core Protein—Subsequent functional analysis used a series of alanine-scanning mutants of the TILRR core protein (supplemental Fig. S1). These showed, as described previously (11), that substitution of Asp-448 caused a 60% reduction in TILRR amplification of inflammatory responses, whereas other mutations had no significant effect (Fig. 2*A*). Parallel experiments to assess the impact of the mutants on cell survival showed that wild type TILRR caused a 42 ± 4% reduction in TUNEL staining. Mutant R425A, which demonstrated wild type levels in regard to inflammatory activation, abrogated the IL-1-induced increase in cell survival. In contrast, effects of other mutants, including D448A, which blocked amplification of inflammatory responses, were indistinguishable from wild type (Fig. 2*B*). FACS analysis demonstrated that cell surface expression of the mutants was comparable with wild type levels (supplemental Fig. S2). We note that a recent entry in the 1000genome database identifies a SNP causing an Asp/Glu mutation of residue 454 in the TILRR sequence (Gene ENSG00000164946, Transcript ID ENST00000380894), corresponding to residue 448 in the shorter, more potent form of the protein (10). Future studies will explore the relevance of this and other TILRR polymorphisms in disease.

The potential impact of the functional substitutions on secondary protein structure was assessed by prediction analysis. This suggested that the mutations caused distinct changes in the α-helical regions of the protein, with no helices predicted in the wild type protein (supplemental Fig. S3). Further, turn and coil regions as well as β regions were comparable with wild type. Such changes, induced by the mutants, are likely to impact IL-1RI complex conformation. Future studies will use crystallography, in combination with surface plasmon resonance and NMR, to determine effects of the functional TILRR mutants on formation and composition of the IL-1RI complex.

Discrete Control of Downstream Responses Correlates with Selective Regulation of MyD88 Recruitment—Subsequent experiments assessed the impact of the mutants on recruitment of the MyD88 adapter. Dominant negative MyD88 reduced amplification of inflammatory genes induced by TILRR and blocked the lower activation levels in the presence of the D448A mutant. In contrast, induction of caspase-3 was unaffected by DNMyD88 (data not shown). Parallel experiments using DNTRAF6 showed reductions in both IL-8 and caspase-3 activities (data not shown).

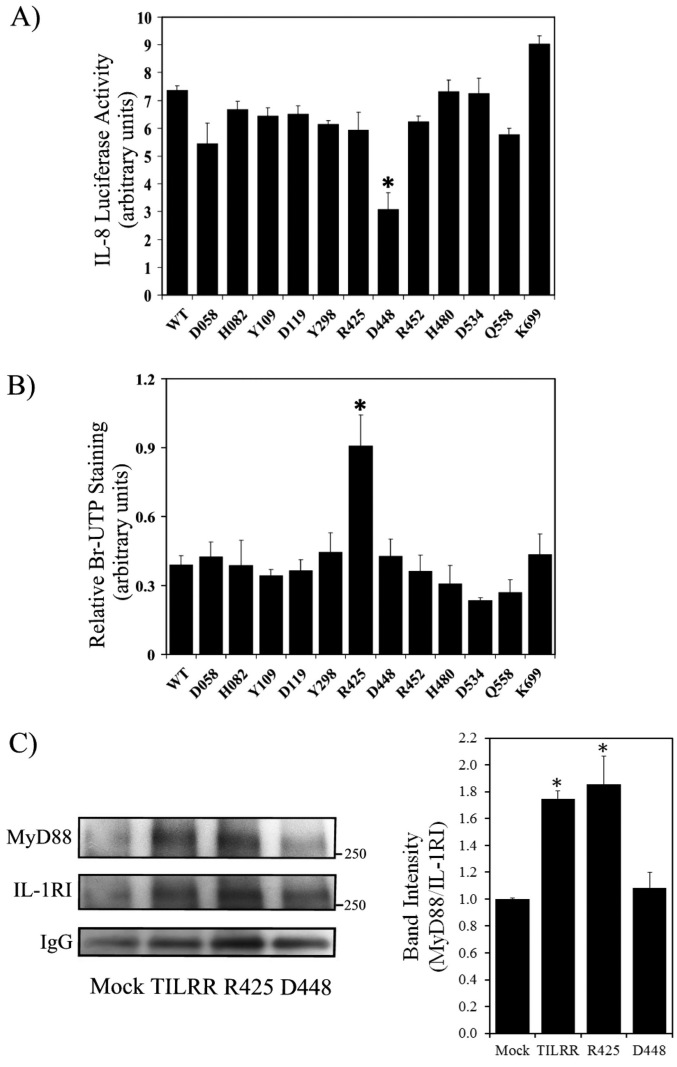

FIGURE 2. **D448A mutant abrogates TILRR-induced inflammatory responses and recruitment of MyD88 to IL-1RI complex.** Cells were grown in a 96-well plate and transfected with empty vector, wild type TILRR cDNA (0.2 μg/ml), or mutant TILRR (0.2–1.2 μg/ml). *A*, cells were co-transfected with pIL-8-Luc (1.25 μg/ml) and TK-RL (0.4 μg/ml), and stimulated with IL-1β (1 nM, 6 h), and luciferase activity was measured. *B*, cells were incubated with IL-1β and treated with TNF and cycloheximide, as in Fig. 1, *B–D*, and stained for BrdUTP. Data are expressed relative to levels in cells transfected with empty vector. Each graph shows the mean ± S.E. of three independent experiments. *, $p < 0.001$. *C*, cells (10^6) transfected with empty vector (*Mock*) or cDNAs containing wild type TILRR or TILRR mutants R425A or D448A (1 μg/ml), and stimulated with IL-1β (1 nM, 20 min), were cross-linked using cross-linker disuccinimidyl suberate (DDS), immunoprecipitated using a polyclonal anti-IL-1RI antibody, separated by SDS-PAGE (4–15%), and levels of MyD88 and IL-1R1 were determined by Western analysis, using IgG as the loading control, as described under "Experimental Procedures". One representative gel is shown. Data are expressed as MyD88/IL-1RI and represent mean ± S.E. of three independent experiments. *, $p < 0.005$.

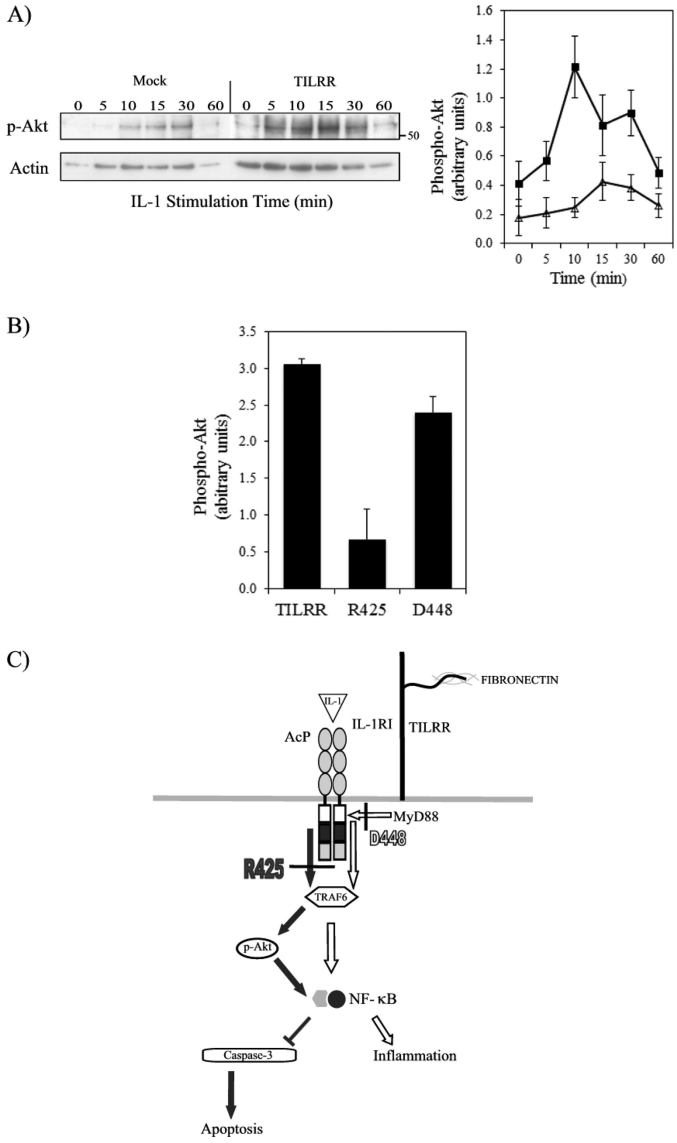

FIGURE 3. **TILRR amplification of Akt phosphorylation is blocked by mutation of Arg-425.** *A*, cells (10^6) were transfected with empty vector (*Mock*, △) or TILRR cDNA (■) (1 μg/ml) stimulated with IL-1β (1 nM) for various times, as indicated, and analyzed by SDS-PAGE (12%). Levels of phospho-Akt (*p-Akt*) were determined by Western analysis as described under "Experimental Procedures," and bands were quantitated using β-actin as the loading control. One representative gel is shown. Data are expressed relative to the loading control and show mean ± S.E. of three independent experiments. $p < 0.01$ at 10 min, and $p < 0.05$ at 15 min. *B*, cells transfected with wild type TILRR or TILRR mutants (R425A or D448A) (1 μg/ml), as indicated, were stimulated with IL-1β (1 nM), and levels of phospho-Akt were determined by Western analysis as in *A*. Data show IL-1-induced activities at 10 min, expressed relative to control (time 0) for each condition, and represent mean ± S.E. of three independent experiments. *, $p < 0.01$. *C*, the D448A mutant blocks TILRR-induced potentiation of MyD88 recruitment and activation of inflammatory genes but has no effect on IL-1-induced Akt phosphorylation and anti-apoptotic signals. In contrast, the R425A mutant abrogates Akt phosphorylation and survival signals, but does not impact TILRR-induced MyD88 recruitment and amplification of inflammatory responses *AcP*, IL-1 receptor accessory protein.

As demonstrated previously (10), TILRR potentiated recruitment of MyD88 to the IL-1RI complex (Fig. 2*C*). The increase was blocked by the D448A mutant, whereas levels in the presence of R425A were the same as induced by wild type TILRR (Fig. 2*C*). The enhanced MyD88 recruitment could promote TILRR amplification by facilitating Myddosome assembly and formation the IL-1RI complex (16–18).

Akt Phosphorylation Is Selectively Regulated through TILRR Mutants—IL-1 stimulation in the presence of TILRR increased activation of the upstream kinase Akt by on average 3-fold (Fig. 3*A*). The effect was abrogated in the presence of the R425A TILRR mutant, whereas substitution of Asp-448 had no impact (Fig. 3*B*). The results are consistent with MyD88-independent control of Akt through the p85/PI3-kinase (19, 20).

In summary, we show that TILRR amplifies IL-1 induced activation of both inflammatory and anti-apoptotic responses

and identify two sites within the TILRR core protein, which selectively control these processes. We demonstrate that alanine substitution of Arg-425 inhibits anti-apoptotic signals and cell survival but amplifies inflammatory responses to levels similar to wild type TILRR (Fig. 3*C*). In contrast, substitution of residue Asp-448 abrogates inflammatory activities, but has no impact on cell survival.

The distinct amplification induced by the mutants is consistent with a role for TILRR in increasing signal specificity, as demonstrated for co-receptors in other systems (21–23). Our data show that this involves selective recruitment of the MyD88 adapter and suggest it to reflect alterations in secondary protein structure and changes in composition and conformation of the signaling receptor complex. The capacity of TILRR to regulate IL-1-induced activities independently makes it a potential therapeutic target for distinct control of inflammatory responses and cell survival.

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REFERENCES

1. Akira, S., and Takeda, K. (2004) Toll-like receptor signaling. *Nat. Rev. Immunol.* **4,** 499–511
2. O'Neill, L. A., and Bowie, A. G. (2007) The family of five: TIR-domain-containing adaptors in Toll-like receptor signaling. *Nat. Rev. immunol.* **7,** 353–364
3. Wang, K. Z., Wara-Aswapati, N., Boch, J. A., Yoshida, Y., Hu, C. D., Galson, D. L., and Auron, P. E. (2006) TRAF6 activation of PI 3-kinase-dependent cytoskeletal changes is cooperative with Ras and is mediated by an interaction with cytoplasmic Src. *J. Cell Sci.* **119,** 1579–1591
4. Barkett, M., and Gilmore, T. D. (1999) Control of apoptosis by Rel/NF-κB transcription factors. *Oncogene* **18,** 6910–6924
5. Susin, S. A., Lorenzo, H. K., Zamzami, N., Marzo, I., Snow, B. E., Brothers, G. M., Mangion, J., Jacotot, E., Costantini, P., Loeffler, M., Larochette, N., Goodlett, D. R., Aebersold, R., Siderovski, D. P., Penninger, J. M., and Kroemer, G. (1999) Molecular characterization of mitochondrial apoptosis-inducing factor. *Nature* **397,** 441–446
6. Wang, W., Prince, C. Z., Mou, Y., and Pollman, M. J. (2002) Notch3 signaling in vascular smooth muscle cells induces c-FLIP expression via ERK/MAPK activation: resistance to Fas ligand-induced apoptosis. *J. Biol. Chem.* **277,** 21723–21729
7. Gay, N. J., and Keith, F. J. (1991) *Drosophila* Toll and IL-1 receptor. *Nature* **351,** 355–356
8. Davis, C. N., Mann, E., Behrens, M. M., Gaidarova, S., Rebek, M., Rebek, J., Jr., and Bartfai, T. (2006) MyD88-dependent and -independent signaling by IL-1 in neurons probed by bifunctional Toll/IL-1 receptor domain/BB-loop mimetics. *Proc. Natl. Acad. Sci. U.S.A.* **103,** 2953–2958
9. McGregor, L., Makela, V., Darling, S. M., Vrontou, S., Chalepakis, G., Roberts, C., Smart, N., Rutland, P., Prescott, N., Hopkins, J., Bentley, E., Shaw, A., Roberts, E., Mueller, R., Jadeja, S., Philip, N., Nelson, J., Francannet, C., Perez-Aytes, A., Megarbane, A., Kerr, B., Wainwright, B., Woolf, A. S., Winter, R. M., and Scambler, P. J. (2003) Fraser syndrome and mouse blebbed phenotype caused by mutations in FRAS1/Fras1 encoding a putative extracellular matrix protein. *Nat. Genet.* **34,** 203–208
10. Zhang, X., Shephard, F., Kim, H. B., Palmer, I. R., McHarg, S., Fowler, G. J., O'Neill, L. A., Kiss-Toth, E., and Qwarnstrom, E. E. (2010) TILRR, a novel IL-1RI co-receptor, potentiates MyD88 recruitment to control Ras-dependent amplification of NF-κB. *J. Biol. Chem.* **285,** 7222–7232
11. Slack, J. L., Schooley, K., Bonnert, T. P., Mitcham, J. L., Qwarnstrom, E. E., Sims, J. E., and Dower, S. K. (2000) Identification of two major sites in the type I interleukin-1 receptor cytoplasmic region responsible for coupling to pro-inflammatory signaling pathways. *J. Biol. Chem.* **275,** 4670–4678
12. Yang, L., Ross, K., and Qwarnstrom, E. E. (2003) RelA control of IκBα phosphorylation: a positive feedback loop for high affinity NF-κB complexes. *J. Biol. Chem.* **278,** 30881–30888
13. Garnier, J., Gibrat, J. F., and Robson, B. (1996) GOR method for predicting protein secondary structure from amino acid sequence. *Methods Enzymol.* **266,** 540–553
14. Garnier, J., Osguthorpe, D. J., and Robson, B. (1978) Analysis of the accuracy and implications of simple methods for predicting the secondary structure of globular proteins. *J. Mol. Biol.* **120,** 97–120
15. Xu, Y., Tao, X., Shen, B., Horng, T., Medzhitov, R., Manley, J. L., and Tong, L. (2000) Structural basis for signal transduction by the Toll/interleukin-1 receptor domains. *Nature* **408,** 111–115
16. Motshwene, P. G., Moncrieffe, M. C., Grossmann, J. G., Kao, C., Ayaluru, M., Sandercock, A. M., Robinson, C. V., Latz, E., and Gay, N. J. (2009) An oligomeric signaling platform formed by the Toll-like receptor signal transducers MyD88 and IRAK-4. *J. Biol. Chem.* **284,** 25404–25411
17. Lin, S. C., Lo, Y. C., and Wu, H. (2010) Helical assembly in the MyD88-IRAK4-IRAK2 complex in TLR/IL-1R signaling. *Nature* **465,** 885–890
18. Gay, N. J., Gangloff, M., and O'Neill, L. A. (2011) What the Myddosome structure tells us about the initiation of innate immunity. *Trends Immunol.* **32,** 104–109
19. Burow, M. E., Weldon, C. B., Melnik, L. I., Duong, B. N., Collins-Burow, B. M., Beckman, B. S., and McLachlan, J. A. (2000) PI3-K/AKT regulation of NF-κB signaling events in suppression of TNF-induced apoptosis. *Biochem. Biophys. Res. Commun.* **271,** 342–345
20. Gustin, J. A., Ozes, O. N., Akca, H., Pincheira, R., Mayo, L. D., Li, Q., Guzman, J. R., Korgaonkar, C. K., and Donner, D. B. (2004) Cell type-specific expression of the IκB kinases determines the significance of phosphatidylinositol 3-kinase/Akt signaling to NF-κB activation. *J. Biol. Chem.* **279,** 1615–1620
21. Lewis, K. A., Gray, P. C., Blount, A. L., MacConell, L. A., Wiater, E., Bilezikjian, L. M., and Vale, W. (2000) β-Glycan binds inhibin and can mediate functional antagonism of activin signaling. *Nature* **404,** 411–414
22. MacDonald, B. T., Yokota, C., Tamai, K., Zeng, X., and He, X. (2008) Wnt signal amplification via activity, cooperativity, and regulation of multiple intracellular PPPSP motifs in the Wnt co-receptor LRP6. *J. Biol. Chem.* **283,** 16115–16123
23. Monje, P. V., Athauda, G., and Wood, P. M. (2008) Protein kinase A-mediated gating of neuregulin-dependent ErbB2-ErbB3 activation underlies the synergistic action of cAMP on Schwann cell proliferation. *J. Biol. Chem.* **283,** 34087–34100