Interleukin (IL-1) plays an important role in inflammation and regulation of immune responses. The activated IL-1 receptor complex, which consists of the IL-1 receptor type I and the IL-1 receptor accessory protein (IL-1RAcP), generates multiple cellular responses including NF-κB activation, IL-2 secretion, and IL-2 promoter activation. Reconstitution experiments in EL4D6/76 cells lacking IL-1RAcP expression and IL-1 responsiveness were used to analyze structure-function relationships of the IL-1RAcP cytoplasmic tail. Mutating a potential tyrosine kinase phosphorylation motif and various conserved amino acid residues had no effect on IL-1 responsiveness. Truncation analyses revealed that box 3 of the TIR domain was required for NF-κB activation, IL-2 production, and c-Jun N-terminal kinase (JNK) activation, whereas IL-2 promoter activation was only partially inhibited. Surprisingly, deletion of aa 527–534 resulted in almost complete loss of all IL-1 responsiveness. Replacement of these aa with alanyl residues did not reconstitute NF-κB activation, IL-2 production, or JNK activation but partly restored IL-2 promoter activation. Immunoprecipitation data revealed a strong correlation between MyD88 binding with NF-κB activation and IL-2 production but not with IL-2 promoter activation. Taken together, our data indicate that box 3 of IL-1RAcP is critical for IL-1-dependent NF-κB activation and stabilization of IL-2 mRNA via JNK, whereas aa 527–534 largely contribute to IL-2 promoter activation.

Interleukin-1 (IL-1) possesses a wide spectrum of inflammatory, metabolic, physiological, hematopoietic, and immunological properties. IL-1 binds to two different receptors of the Ig superfamily: IL-1 receptor type I (IL-1RI) and IL-1 receptor type II (IL-1RII). Both receptors bind the ligands with distinct affinities (1). Whereas IL-1RI is necessary for signal transduction (2), IL-1RII is not capable of transducing an activation signal but rather acts as a decoy receptor serving as a ligand sink and competing for IL-1 with the IL-1RI (3–5). An additional member of the IL-1 receptor family, IL-1 receptor accessory protein (IL-1RAcP), has also been identified. The 66-kDa IL-1RAcP shares limited homology with IL-1RI and IL-1RII but does not bind IL-1 (6). It has been shown that co-expression of the IL-1RAcP is essential for a fully functional IL-1RI complex (7–10).

In the last years the elucidation of early IL-1 signaling events has progressed. After ligand-induced complex formation of IL-1RI and IL-1RAcP (7–11), the Ser/Thr kinase IRAK is recruited to the receptor complex, where it becomes highly phosphorylated (12, 13). Recruitment of IRAK requires the intracellular domains of both receptor chains (8, 10, 14). Dominant-negative forms of MyD88 block IL-1 signaling (15). After phosphorylation by itself (12) or by additional kinases (16), IRAK leaves the receptor complex and interacts with tumor necrosis factor receptor-associated factor 6 (17). Recently, the MAP kinase kinase kinase TAK1 has been identified to interact with tumor necrosis factor receptor-associated factor 6 in association with TAB1 and TAB2 (18–22), thereby providing a link to the machinery that activates nuclear factor κB (NF-κB) via stimulation of IκB kinases (IKKα and IKKβ) (23–28). One critical component in the signaling cascade is MyD88 (29, 30), because mice lacking this adaptor molecule do not show cytokine-induced activation of NF-κB and c-Jun N-terminal kinase (JNK) (31).

We have previously described an IL-1RI-positive subclone of EL4 cells, EL4D6/76, which binds IL-1 with high affinity but fails to activate NF-κB or produce IL-2 following IL-1 stimulation (32). This defect is due to the lack of IL-1RAcP expression and can be overcome by transfection with IL-1RAcP, thus reconstituting IL-1-specific functional defects in EL4D6/76 cells (7, 9). In the present study we investigated regions within the cytoplasmic domain of IL-1RAcP that are required for perpetuating IL-1 responses. We present data demonstrating that, in addition to box 3 (aa 538–542) of the TIR domain, aa 527–534 within the cytoplasmic domain of IL-1RAcP are essential for IL-1 signaling.

**EXPERIMENTAL PROCEDURES**

*Construction of Truncated and Mutated Forms of IL-1RAcP—Truncated forms of IL-1RAcP were constructed by PCR technique. The vector pEF-IL-1RAcP containing the murine IL-1RAcP coding sequence was used as a template (9). Truncated fragments were cloned into pFLAG-IL-1RAcP (kind gift of Michael Martin, Hannover, Germany), a
derivative of pFLAG-CMV-1 (Sigma). Deletions in the IL-1RAcP sequence were performed with the ExSite™ PCR-based site-directed mutagenesis kit (Stratagene, Amsterdam, The Netherlands), whereas point mutations were generated by site-directed mutagenesis using the QuickChange™ site-directed mutagenesis kit (Stratagene) according to the manufacturer’s recommendation. The cDNA was cloned into the pFLAG-IL-1RAcP using BgII and XbaI, respectively. All truncations and mutations were verified by sequence analysis.

**Construction of Reporter Plasmids**—An IL-1-inducible fragment of the murine IL-2 promoter (−1 to −303) was cloned into KpnI and BgII into the luciferase reporter vector pGL3-Basic (Promega, Mannheim, Germany) (pGL3-IL-2−303) (33). For determination of transcriptional activity of NF-κB, five tandemly arranged NF-κB binding sites from the HIV long terminal repeat enhancer (5′-GGGACATTTC-3′) (34) were cloned via BgII and HindIII into the luciferase reporter vector pGL3-Basic (pGL3-5xNFκB).

**Cell Culture and Biological Reagents**—Mouse thymoma EL4 D6/76 cells were cultured in RPMI 1640 (Pan Biotech, Aidenbach, Germany) containing 2 mM glutamine, 10% fetal calf serum, 100 units/ml penicillin, 100 μg/ml streptomycin, and 30 μg 2-mercaptoethanol (culture medium) at 37°C in humidified air with 5% CO₂. For stimulation, 2 × 10⁵ cells were seeded in 96-well plates at a density of 1 × 10⁶ cells/ml. Recombinant human IL-1α (rhIL-1α) and recombinant mouse IL-1β (mIL-1β) were purchased from R&D Systems GmbH (Wiesbaden, Germany), whereas PMA was purchased from Sigma. Human embryonic kidney 293 cells were cultured and stimulated in Dulbecco’s modified essential medium high glucose (PAA Laboratories, Linz, Austria) containing 10% fetal calf serum, 100 units/ml penicillin, 100 μg/ml streptomycin under the conditions indicated above.

**Transfection of EL4D6/76 and 293IL-1RI Cells**—EL4D6/76 cells at a density of 5 × 10⁵/ml were transiently transfected by the DEAE-dextran method. Briefly, the transfection reagent was freshly prepared by mixing 300 μl of 0.5% DEAE-dextran in Tris-buffered saline (25 mM Tris-HCl, pH 7.4, 123 mM NaCl, 5 mM KCl, 0.7 mM CaCl₂, 0.5 mM MgCl₂, 0.6 mM Na₂HPO₄) and 300 μl of chloroquine (80 μg/ml in Tris-buffered saline) containing 500 ng of IL-1RAcP expression plasmid encoding the various mutated forms of the co-receptor chain together with 1 μg of the reporter plasmid. 5 × 10⁵ cells (in logarithmic growth phase) were washed in PBS, pH 7.4, and resuspended in Tris-buffered saline. After centrifugation the supernatant was decanted, and the cells were resuspended in the residual liquid. Subsequently, the DEAE-dextran-DNA transfection mix was added, and the cells were incubated for 30 min at room temperature. Thereafter, the cells were washed twice with cold red-free RPMI 1640 medium (Pan Systems) plus supplements and cultured for 24 h in 6-well plates (Costar, Cambridge, MA) at a density of 1 × 10⁶ cells/ml. 293IL-1RI cells stably expressing human IL-1RI (12) were co-transfected by the calcium phosphate precipitation method with different amounts of pRKT-Myc/MdD88 encoding human Myd88 fused to an N-terminal Myc tag and 1–3 μg of pFLAG-IL-1RAcP encoding mouse wild type (wt) or mutant co-receptor chain, respectively. The presence of DNA in all transfections was kept constant by adding empty vector cDNA.

**Detection of Luciferase Activity in Transiently Transfected Cells**—For determination of the IL-2 promoter activation or NF-κB activation, EL4D6/76 cells were co-transfected with the luciferase reporter plasmids pGL3-IL-2−303) or pGL3-5xNFκB, respectively. After cultivation for 18 h, 2 × 10⁵ transfected cells were seeded per well of a ViewPlate-96 (Packard, Groningen, The Netherlands) at a density of 1 × 10⁶/ml in RPMI 1640 without phenol red and stimulated in the absence or presence of 20 units/ml rhIL-1α, 250 ng/ml rhIL-1β, 10 ng/ml PMA or co-stimulated with PMA and cytokine for 18 h. Luciferase activity was detected by the Steady-Glo luciferase assay kit (Promega, Mannheim, Germany) according to the manufacturer’s instructions. As an internal control, EL4 D6/76 cells were co-transfected with 300 ng of pRL-SV40 vector (Promega) encoding Renilla luciferase under control of SV40 early enhancer/promoter leading to constitutive expression of Renilla luciferase. Expression of both firefly and Renilla luciferase was detected using the dual luciferase reporter assay system (Promega) as described by the manufacturer. The luciferase activity was measured in at least triplicate cultures for 0.1 min using the TopCount microplate scintillation counter (Packard).

**IL-2 Enzyme-linked Immunosorbent Assay**—IL-2 secretion into the culture supernatants was quantified by sandwich enzyme-linked immunosorbent assay. Microwell plates were coated with rat anti-mouse IL-2 (Pharmingen, Hamburg, Germany; 1 μg/ml in 0.1 M NaHCO₃, pH 8.5). Additional binding sites were blocked with PBS with 10% fetal calf serum, and the plates were washed with PBS with 0.05% Tween 20. After addition of 50 μl of culture supernatants or serial diluted recombinant proteins as standards, the plates were incubated for 90 min at 37°C, washed extensively, and incubated with biotinylated rat anti-mouse IL-2 (Pharmingen; 1 μg/ml in PBS with fetal calf serum) for a further 60 min at 37°C. After extensive washing, the plates were incubated with 100 μl of a streptavidin-peroxidase conjugate (Roche Molecular Biochemicals) for 30 min at 37°C followed by the addition of 100 μl of a 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid solution (Devitron, Castrop-Rauxel, Germany). The signals were detected by measuring the extinction at 405 nm against a reference wavelength at 490 nm using the Titertek Multiskan MCC microplate reader (Flow, Meckenheim, Germany). Quantification of IL-2 in the samples derived from a standard curve obtained with the recombinant cytokine.

**Immunoprecipitation and Western Blotting**—24–36 h after transfection, 293II-1RI cells were stimulated in the presence or absence of 50 ng/ml rhIL-1α (Genentech, San Francisco, CA) for 5 min and lysed in 1 ml of lysis buffer (0.5% Nonidet P-40, 250 mM NaCl, 1 mM EDTA, 20 mM β-glycerophosphate, 1 mM NaVO₃, 5 mM p-nitrophenyl phosphate, 1 mM dithiothreitol, 50 mM HEPES, pH 7.9, supplemented with protease inhibitor mixture; Roche Molecular Biochemicals). The cleared lysates were adjusted to 0.75% Nonidet P-40 and 375 mM NaCl, divided into two aliquots, and incubated with either preimmune serum and protein A (Amersham Biosciences) or anti-FLAG M2-Sepharose (Sigma) for 1 h at 4°C. After thorough washing, 160 milliunits/ml streptavidin-peroxidase conjugate (Sham Biosciences) was added and the samples were incubated with 160 milliunits/ml streptavidin-peroxidase conjugate (Sham Biosciences). The signals were separated by 4–20% SDS-PAGE and visualized by Western blotting using the ECL kit (Amersham Biosciences).

**Detection of JNK Activity**—5 × 10⁵ EL4D6/76 cells were transfected as indicated with 1 μg of IL-1RAcP expression plasmid encoding the various mutated forms of the co-receptor chain together with 500 ng of pGL3-Control vector (Promega) constitutively expressing the luciferase reporter gene. After a recovery phase of 24 h at 37°C, the cells were starved for 24 h in RPMI 1640/PBS (1:1) supplemented with 10 mM HEPES, antibiotics, and 2-mercaptoethanol and divided into two aliquots. One aliquot was used for the determination of luciferase activity and the other was added to 1% Nonidet P-40 and 500 mM NaCl and boiled in Laemmli buffer (35). The solubilized proteins were separated by 4–20% SDS-PAGE and visualized by Western blotting using the ECL kit (Amersham Biosciences).

**Statistical Analysis**—Statistical differences between the mean values were analyzed using the two-sided Student’s t test.

**RESULTS**

In addition to IL-1RI, co-expression of the IL-1RAcP is essential for a fully functional receptor complex (7–10). Therefore we investigated the contribution of the cytoplasmic domain of IL-1RAcP to IL-1-induced signal transduction. To test the functionality of truncated or mutated IL-1RAcP constructs, EL4D6/76 cells lacking IL-1RAcP expression (9) were transiently transfected with these constructs, and IL-1-mediated signal transduction was investigated in the transfectants using the co-transfected luciferase reporter pGL3-IL-2−303).

Arbitrary stepwise truncations showed that the carboxy terminus of murine IL-1RAcP protein could be truncated by 19 aa, up to aa 551 without affecting its capacity to activate the IL-2 promoter. However, truncation for an additional 40 aa, up to residue 511, as well as truncation of the complete cytoplasmic domain, up to aa 384, resulted in a complete loss of function (data not shown). More detailed truncation studies (Fig. 1, A and B) demonstrated that IL-1RAcP constructs truncated up to aa 544 showed a significantly enhanced activation of the IL-2
Fig. 1. Effect of truncation mutants of IL-1RαC on IL-1 responsiveness. A, schematic representation of the truncated IL-1RαC constructs. The open bars represent the extracellular domains of IL-1RαC, the black boxes represent the transmembrane domains (TM), and the gray boxes represent the cytoplasmic domains (CD) of IL-1RαC. The numbers in bold type represent aa positions in the mature protein. B, IL-2 promoter activation. C, NF-κB activation. D, IL-2 production.
**Functional Analysis of IL-1RaCP Intracellular Domain**

**Fig. 2. Effect of different mutant variants of IL-1RaCP on IL-1-induced IL-2 production and IL-2 promoter activation.**

A, partial aa sequence of wt and mutant IL-1RaCP. B, EL4D6/76 cells were transiently transfected with pGL3-IL-2(530–303) together with the indicated IL-1RaCP constructs and stimulated as described in the legend to Fig. 1. After stimulation IL-2 promoter activation and IL-2 secretion were detected as described. The data are expressed as the means ± S.E. of three to nine separate experiments performed at least in triplicate. *, p < 0.001 compared with wt; **, p < 0.001 compared with IL-1RaCP Δ527–534.

**Table:**

| IL-1RaCP Mutants | IL-2 Promoter Activation | IL-2 Production |
|------------------|--------------------------|-----------------|
| 8 x Ala          |                          |                 |
| Δ527–533         |                          |                 |
| Δ528–534         |                          |                 |
| Δ528–533         |                          |                 |
| Δ527–534         |                          |                 |
| K525R/E529D/ Y535F |                          |                 |
| W526F/K527G      |                          |                 |
| P534A            |                          |                 |
| WT               |                          |                 |

Promoter activation in a luciferase reporter assay. EL4D6/76 cells were transiently transfected with 1 μg of luciferase reporter under control of the IL-1-inducible IL-2 promoter (pGL3-IL-2(530–303)) and 500 ng of the indicated IL-1RaCP constructs as described under “Experimental Procedures.” 24 h after transfection, the cells were stimulated in the absence or presence of 10 units/ml rhIL-1α, 250 ng/ml rmIL-18, 10 ng/ml PMA, or a combination of cytokine + PMA. After incubation for an additional 18 h, luciferase activity was determined in triplicate as a measure of IL-2 promoter activation, and stimulation indices were calculated and normalized to the PMA response. NF-κB activation (C) as well as IL-2 production (D) were measured after co-transfection of EL4D6/76 cells with 500 ng of the indicated IL-1RaCP constructs and 1 μg of reporter plasmid (pGL3-5×NFκB) containing five NF-κB binding sites. For determination of NF-κB activation, the transfectants were stimulated with 10 units/ml rhIL-1α, 250 ng/ml rmIL-18 or left untreated. D, for induction of IL-2 secretion, the cells were stimulated with IL-1 or IL-18 in the absence or presence of 10 ng/ml PMA for additional 18 h. After stimulation the luciferase activity was calculated as a measure of NF-κB activation, and IL-2 secretion into the supernatants was detected by sandwich enzyme-linked immunosorbent assay. The data represent the means ± S.E. of three to six separate experiments performed in at least triplicates. *, p < 0.001 compared with wt; **, p < 0.001 compared with IL-1RaCP Δ523. The numbers given in the figure represent the percentages of the wt response.
capacity. Deletion of aa 524–537 resulted in the inability of the protein to activate both IL-1-specific effects, namely IL-2 production and IL-2 promoter activation. Similar results were obtained with mutant IL-1RACP molecules lacking aa 524–551 (data not shown). In this context, aa 527–534 were found to be the shortest region essential for IL-1 responsiveness, because deletion of these aa resulted in an almost complete loss of IL-1-induced IL-2 promoter activation and IL-2 production compared with wt IL-1RACP (9.1 and 2.3% residual activity, respectively). Smaller deletions (i.e. Δ528–533, Δ528–534, and Δ527–533) had no effect on the IL-1 response (Fig. 2). Replacement of aa 527–534 by 8 alanine residues (8 × Ala) did not reconstitute IL-1-induced IL-2 production but did partly restore IL-2 promoter activation capacity (24% of the wt response; Fig. 2), suggesting that for IL-2 promoter activation a synergistic contribution of aa 527–534 and box 3 is necessary for optimal effects. Single aa changes of the conserved Pro534 → Ala as well as mutation of conserved Trp526 → Phe and the neighboring Lys527 → Gly did not affect IL-1 responsiveness (Fig. 2). Furthermore, the critical region was found to harbor a potential tyrosine kinase phosphorylation motif. However, mutation of the three consensus aa Lys527, Glu529, and Tyr535 to Arg, Asp, and Phe had no effect on either IL-1-induced IL-2 promoter activation and IL-2 production (Fig. 2).

To further validate our data, EL4D6/76 cells were co-transfected, in addition to the pGL3-IL-2 (−303) reporter construct and selected IL-1RACP mutants, with a Renilla luciferase reporter construct leading to constitutive expression of Renilla luciferase. After stimulation, the data on IL-2 promoter-driven firefly luciferase activity confirmed our results observed in the single luciferase reporter assay, whereas Renilla luciferase activity was identical in all lysates, indicating that experimental conditions were comparable in each case analyzed (data not shown).

Furthermore, we examined the effect of IL-1 on activation of the stress-activated protein kinase pathway as another major downstream signaling event. For this purpose, EL4D6/76 cells were transiently transfected with various mutants of IL-1RACP and analyzed for IL-1-dependent activation of JNK. As demonstrated in Fig. 3., mutants of IL-1RACP lacking box 3 (i.e. IL-1RACPΔ523 and IL-1RACPΔ537) did not show any IL-1-dependent activation of JNK. In contrast, in the presence of box 3 (IL-1RACPΔ544) JNK activation occurred, although it was reduced to ~65% of the wt response. Deletion of aa 527–534 resulted in the complete loss of IL-1-induced JNK activation. Substitution of this aa stretch by 8 alanine residues (8 × Ala) was unable to reconstitute IL-1 responsiveness.

Because we found aa 527–534 to be essential for IL-1 responsiveness, we speculated that this region might be crucial for the recruitment of downstream adapter molecules, such as MyD88, to the receptor complex. To verify this hypothesis, 293 cells stably expressing IL-1RI (293IL-1RI) were transiently transfected with MyD88 fused to a Myc tag and a combination of wt or mutant IL-1RACP, the latter expressed as a fusion protein with a FLAG tag at the N terminus. After stimulation of the transfectants, IL-1RACP was immunoprecipitated, and MyD88 was detected by Western blotting. As shown in Fig. 4, MyD88 could only be detected after co-transfection with wt IL-1RACP. In other co-expression/co-precipitation experiments with 293 cells, mutations of the conserved Trp526 → Phe and the neighboring Lys527 → Gly as well as mutation of the tyrosine kinase phosphorylation motif within IL-1RACP did not adversely affect binding of MyD88 (data not shown). In the absence of box 3 of the TIR domain (IL-1RACPΔ523 and IL-1RACPΔ537), no binding of MyD88 could be observed. Even in the presence of box 3, no MyD88 binding was found after deletion of aa 527–534 or after their replacement by 8 alanine residues. As control, expression of MyD88 was analyzed in total cell lysates and was found to depend on the amount of DNA used for transfection.

**DISCUSSION**

We have shown previously that co-expression of IL-1RI and IL-1RACP is essential for IL-1-mediated signaling (7–10). In the present study we investigated the role of the cytoplasmic domain of IL-1RACP in IL-1 signaling by generation of truncation and deletion mutants lacking certain regions within the cytoplasmic tail.

Our data provide evidence for the existence of different functional motifs within the cytoplasmic part of IL-1RACP, because IL-2 promoter activation, IL-2 production, and NF-κB activation as well as JNK activation are differentially affected by the mutations. After truncation of 40 aa up to residue 530, IL-2 promoter activation is still detectable, whereas very little IL-2 production can be observed, indicating that both IL-1-induced
effects may be differentially controlled, although one would expect that IL-2 promoter activation would correlate with IL-2 production. IL-1RAcP harbors a C-terminal homology motif of the TIR domain highly conserved among the members of the Toll/IL-1 receptor family, called box 3 (aa 538–542) (Fig. 5, top panel) (39). As demonstrated by our data, in the absence of box 3 only very little NF-κB activation, IL-2 production, or JNK activation could be detected, indicating that this motif is required for activation of the transcription factor, the stress-activated protein kinase and eventually for IL-2 production. Heguy et al. (2) have shown previously that box 3 is also required in the IL-1RI, so that for successful IL-1 function box 3 must be present in both chains. It has been shown previously that MAP/stress-activated protein kinases such as JNK are implicated in IL-2 mRNA stabilization (40) and that neither IRAK nor tumor necrosis factor receptor-associated factor 6 participates in this process, which is required for effective IL-2 protein production (41). In a previous report, CD28 activation has been found to stabilize IL-2 mRNA (42) and to up-regulate JNK activity (43), suggesting that JNK plays a pivotal role in IL-2 mRNA stabilization directly or eventually via an activation of the transcription factors ATF and AP-1 (44–46) as is also demonstrated by our data. Recently, it has been shown that p42/p44 and p38 MAP kinase are involved in IL-1-induced induction of IL-2 in the murine thymoma cell line EL4 NOB-1. The target for p42/p44 MAP kinase might be transcription, whereas p38 MAP kinase is likely to be targeting post-transcriptional processes (47). Furthermore, Winzen et al. (48) demonstrated an important role of the p38 MAP kinase pathway in regulation of mRNA stability via MK-2. We predict that this pathway is also dependent on box 3. Recently, the effect of mutations within the cytoplasmic region of IL-1RI on different signaling functions including activation of NF-κB and stress kinases was examined and demonstrated the involvement of boxes 1 and 2 but not box 3 (49). Thus, at least for the tested responses in the heterodimeric complex synergistic actions of box 3 within IL-1RAcP and boxes 1 and 2 in IL-1RI are required.

As an early signaling event, IRAK is recruited to the IL-1R complex (15). Recruitment of IRAK to IL-1RAcP is mediated by a recently described scaffolding protein, called Tollip, interacting with IL-1RAcP (50). More recently, Tollip was shown to be phosphorylated by IRAK upon stimulation with IL-1 (51). Tollip/IRAK was found associated with IL-1RAcP (aa 538–542) of IL-1RAcP, critical for IL-1-dependent NF-κB activation and stabilization of the IL-2 mRNA via JNK, whereas aa 527–534 as constituents of the neighboring loop show functional activity for IL-2 promoter activation in the absence of box 3 (Fig. 5B). As shown, full IL-1 responsiveness requires synergistic function of box 3 and aa 527–534 (Fig. 5D). Deletion of aa 527–534 removes the C-terminal loop and alters the position of box 3 toward the rest of the molecule affecting its function and destroying the synergism (Fig. 5A). The 8 x Ala substitution enables correct repositioning of box 3 allowing partial IL-2 promoter activation possibly mediated by an adapter different from MyD88. This putative adapter synergizes with aa 527–534 to yield full IL-2 promoter activation (Fig. 5C). Binding of MyD88 to the IL-1R complex is required for JNK and NF-κB activation. This binding in our model was strictly dependent on aa 527–534. We can
only speculate about the function of this region. It could be responsible for the heterodimeric interaction or for the binding of an adapter like Tollip, which might participate in IL-2 promoter activation and mediate binding of MyD88. Efficient NF-kB activation and IL-2 production correlated with the detectable presence of MyD88 in the activated receptor complex, whereas IL-2 promoter activation was much less dependent on its presence. The differential effects on IL-2 promoter and NF-kB activation might reflect the distinct activation requirements for the IL-2 promoter and the NF-kB-responsive element. We have shown previously that IL-1 acts on the IL-2 promoter by activating the T cell element distal element via IL-1R and mitogens and not by IL-1 alone, whereas NF-kB transcriptional activity can already be activated by a single stimulus (IL-1) (33).

Taken together, our data indicate the existence of regions with functional variations within the cytoplasmic part of IL-1RαCp. The identification of these regions should enable us to develop new strategies for the intervention of IL-1 signaling crucial for several immunological processes.

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