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The Type II IL-1 Receptor Interacts with the IL-1 Receptor Accessory Protein: A Novel Mechanism of Regulation of IL-1 Responsiveness

Detlef Lang,* Johannes Knop,* Holger Wesche,2* Ute Raffetseder,* Roland Kurrle,‡ Diana Boraschi,‡ and Michael U. Martin3* 

IL-1 binds to two types of receptors on the cell membrane, of which only type I (IL-1RI) transduces signals in concert with the coreceptor IL-1 receptor accessory protein (IL-1RAcP) while type II (IL-1RII) allegedly functions solely as ligand sink and decoy receptor without participating in IL-1 signaling. To investigate the regulatory role of IL-1RII on IL-1 responsiveness, a chimeric receptor encompassing the extracellular and transmembrane portions of IL-1RII and the cytoplasmic signal-transducing domain of IL-1RI was transfected into two murine EL-4-derived sublines that do or do not express IL-1RAcP, respectively. The chimeric receptor was able to transduce the IL-1 signal and induce IL-2 production only in the cell line which expressed IL-1RAcP, suggesting effective interaction between the extracellular domains of IL-1RII and IL-1RAcP in the presence of IL-1. The physical association of ligated IL-1RII with IL-1RAcP was proven by crosslinking experiments with radio-iodinated IL-1 and subsequent immunoprecipitations in normal human B cells and in EL-4 D6/76 cells transiently cotransfected with IL-1RII and IL-1RAcP, respectively. Based on these findings, it is proposed that upon IL-1 binding IL-1RII can recruit IL-1RAcP into a nonfunctional trimeric complex and thus modulate IL-1 signaling by subtracting the coreceptor molecule from the signaling IL-1RI. In this novel mechanism of coreceptor competition, the ratio between IL-1RII and IL-1RI becomes the central factor in determining the IL-1 responsiveness of a cell and the availability of IL-1RAcP becomes limiting for effective IL-1 signaling. The Journal of Immunology, 1998, 161: 6871–6877.

Interleukin-1 is a potent mediator of the immune system which regulates development and sustenance of inflammatory responses. An excessive or prolonged release and action of this cytokine at the site of inflammation can have pathologic consequences, as its catabolic properties, necessary at the beginning of an immune response to mobilize defense mechanisms, can eventually become detrimental to the host. One example is the deleterious role of IL-1 in rheumatoid arthritis, where it ultimately leads to destruction of the joint (2). Nature impressively demonstrates that this powerful cytokine system requires a tight network of regulation by providing the IL-1 receptor antagonist (IL-1Ra), presently the only known naturally occurring true receptor antagonist (3).

IL-1 effects are mediated by specific plasma membrane receptors. Two closely related types of receptors for IL-1 have been cloned (4, 5), of which type I IL-1 receptor (IL-1RI) transduces signals into the target cell (6), whereas the function of a ligand sink or decoy receptor was ascribed to type II IL-1 receptor (IL-1RII) (7). IL-1RI binds IL-1α or IL-1β through its extracellular domain and then interacts with the IL-1 receptor accessory protein (IL-1RAcP) (8) to form a functional signaling receptor complex (9, 10). On the other hand, binding of IL-1Ra to IL-1RII does not recruit IL-1RAcP, thus preventing formation of the signaling complex (8). The ligand-induced association of the two transmembrane molecules allows the interaction of the cytoplasmic portions of receptor and coreceptor necessary for the association of the adapter protein MyD 88 and the activation of the IL-1 receptor associated protein kinases (IRAK) (11), which subsequently leads to the activation of the transcription factor NF-κB (12, 13). Recently, a second member of the IRAK family, termed IRAK-2, was cloned that is able to interact with MyD 88 and the IL-1RII chain upon overexpression in manner comparable to IRAK (14). As the extracellular domains which bind the ligands IL-1α, IL-1β, or IL-1Ra are rather homologous in IL-1RI and IL-1RII, it was tempting to speculate that, upon IL-1 binding, IL-1RII would also interact with IL-1RAcP.

Here we show that a chimeric receptor consisting of the extracellular and transmembrane portion of human IL-1RII (hIL-1RII) plus the signaling cytoplasmic tail of IL-1RII (15) can only signal in the presence of IL-1RAcP. Furthermore, we demonstrate the direct interaction of hIL-1RII with murine IL-1RAcP (mIL-1RAcP) by crosslinking and immunoprecipitation studies. These results prove the physical interaction of ligand-bound IL-1RII with IL-1RAcP. The fact that IL-1RAcP can interact with both types of ligated IL-1 receptors allows to propose the novel mechanism of coreceptor competition and suggests that down-regulation of IL-1 responsiveness is possible through sequestration of IL-1RAcP by IL-1RII.
Materials and Methods

Mammalian cell culture

Mammalian cell culture was conducted at 37°C, 5% CO2. Murine EL-4 cells were maintained in RPMI 1640 supplemented with 5% (v/v) FCS, 1 mM pyruvate, nonessential amino acids (MEM), and 2 mM l-glutamine. Media were from BioWhittaker (Boehringer Ingelheim Bioproducts, Heidelberg, Germany) and supplements from Life Technologies (Eggenstein, Germany). EL-4 D6/76 cells (16) were a kind gift of W. Falk (Regensburg, Germany). EL-4 AcP1 is a clone derived from EL-4 D6/76, which overexpresses murine IL-1RAcP as described previously (10).

Plasmids and generation of stable cell lines and transient transfections

The expression plasmid pSV77, encoding the IL-1R chimera (amino acids 1–369 of hIL-1RII and amino acids 340–552 of hIL-1RI; Ref. 15) was a kind gift of G. Macchia (L’Aquila, Italy). The expression plasmid pMIM38 encoding full-length cDNA for hIL-1RII has also been described (17). A complete cDNA for murine IL-1RAcP was generated from the vector pEF-Acp (10) by PCR cloning and inserted into the KpnI/NcoI sites of the expression vector pFLAG-CMV-1 (Kodak, New Haven, CT). The plasmid pBape Puro (18) encoded a puromycin resistance gene was kindly provided by B. Lüscher (Hannover, Germany).

To generate stable cell lines expressing the chimeric IL-1R EL-4 AcP1 cells (AcP1) or EL-4 D6/76 cells were cotransfected with pSV77 and pBape Puro by electroporation as described previously (10). Transfected cells were maintained in medium containing penicillin/streptomycin and 2 µg/ml puromycin (Sigma, Deisenhofen, Germany) for 2 wk. Single cell clones were isolated from puromycin-resistant pools by limiting dilution (0.3 cell/well) and screened for expression of chimeric mRNA using RTPCR. From 13 AcP1-derived clones 1 expressed mRNA for the chimera, and from 58 EL-4 D6/76-derived clones 3 expressed mRNA for the chimera.

Transient transfection of EL-4 D6/76 cells was performed using DEAE-dextran as described (19) with minor modifications. In summary, cells were cultured overnight at a density of 2 × 106 cells/ml. Before transfection, cells were washed twice with PBS and once with TBS (25 mM Tris-HCl (pH 7.4), 137 mM NaCl, 5 mM KCl, 0.6 mM Na2HPO4, 0.7 mM CaCl2, 0.5 mM MgCl2). Cells (5 × 106) were resuspended in a transfection solution containing a total of 0.5 µg DNA, 250 µg/ml DEAE-dextran (Pharmacia, Freiburg, Germany), and 40 µg/ml Chloroquine (Sigma) in TBS. Equal amounts of DNA were guaranteed by adding pcDNA3 or pFLAG-CMV-1, respectively. Transfection was conducted for 30 min at room temperature. After incubation cells were washed twice with medium and cultured for 18–24 h in 6-well plates.

Induction and quantitation of IL-2 production

Cells were seeded at a density of 5 × 105 cells/well in microtiter plates with medium containing the calcium ionophore A23187 (Sigma, 2.5 × 10−7 M) and different concentrations of IL-1 (kind gift of J. Sims, Immunex, Seattle), blocking anti-murine IL-1RI mAb (35F5, Pharmingen, Hamburg, Germany), blocking anti-human IL-1RI mAb (Genzyme, Cambridge, MA), and an IL-1Ra munit (Doob 0039; Ref. 20) as indicated in the figures. After 18 h, supernatants were removed and IL-2 was measured using the DuoSet for mouse IL-2 according to the manufacturer’s instructions (Genzyme).

Crosslinking of IL-1R components

To investigate the physical interaction of IL-1RI with IL-1RAcP, EL-4 D6/76 cells were transfected with various combinations of expression plasmids as indicated in the figures. Radiolaabeled [32P]IL-1α was prepared with a standard chloramine-T method. Transfected cells (2 × 106) were incubated with 10–20 ng/ml [32P]IL-1α for 3 h. For blocking of endogenous IL-1RI, 2 µg/ml of the mAb 35F5 (Pharmingen, Hamburg, Germany), blocking anti-human IL-1RI mAb (Genzyme, Cambridget, MA), and an IL-1Ra munit (Doob 0039; Ref. 20) as indicated in the figures. After 18 h, supernatants were removed and IL-2 was measured using the DuoSet for mouse IL-2 according to the manufacturer’s instructions (Genzyme).

Immunoprecipitations

Murine IL-1RI was precipitated from the lysates using the nonblocking mAb 12A6 (PharMingen) at a concentration of 2 µg/500 µl lysates from 2 × 106 cells. Binding of the mAb was overnight at 4°C before 50 µl of a protein G-Sepharose slurry (Pharmacia) were added for further 4 h. Immunoprecipitates were washed twice with lysis buffer and then prepared for SDS-PAGE by heating with 50 µl of Laemmli buffer.

FLAG-tagged IL-1RAcP was precipitated from lysates with 25 µl of anti-FLAG-M2 affinity gel (Kodak, New Haven, CT) overnight. Immunoprecipitates were washed four times with a high salt buffer (1% Nonidet P-40, 1% sodium deoxycholate, 0.1% SDS, 0.25 M NaCl, 0.01 M sodium phosphate (pH 7.0), 2 mM EDTA). FLAG-IL-1RAcP was released from the M2 Ab by adding an excess of FLAG peptide (100 µg/ml; Kodak) for 30 min. For precipitation of the hIL-1RII, these supernatants or lysates were incubated with 5 µg of a nonblocking anti hIL-1RII mAb (R.K., unpublished data) for 2 h at 4°C. Then 10 µg per 500 µl/sample of a goat anti-mouse Ig Fc Ab (Dianova, Hamburg, Germany) were added and incubated overnight. The immune complexes were precipitated with 50 µl of a protein G-Sepharose slurry (Pharmacia). Precipitates were washed four times with high salt buffer and analyzed as described above. Isotype control of the second immunoprecipitation was performed with the mouse IgG2a mAb OXK3.

Detection of IL-1RII/IL-1RAcP complexes in human B cells/monocytes

Human B cells and monocytes were prepared from buffy coats obtained from the blood bank of the Medical School by a standard protocol. Erythrocytes and neutrophils were removed by a Ficoll separation. T cells were depleted by rosetting with erythrocytes from sheep, and the remaining cells, mainly B cells and monocytes, were cultured overnight in RPMI 1640 + 5% FCS. Nonadherent cells were removed and washed twice with PBS. Binding and crosslinking of [3H]IL-1α were performed as described for EL-4 cells.

For immunoprecipitation of hIL-1RAcP a rabbit antiserum was used (kindly provided by Dr. Z. Cao, Tularik, San Francisco). Lysates and immunoprecipitates were analyzed as described above.

Results

The aim of this study was to demonstrate the interaction between IL-1RII and IL-1RAcP in the presence of IL-1. In contrast to the interaction of IL-1RI with IL-1RAcP, formation of such a complex would not result in signal transduction because IL-1RII lacks the cytoplasmic “Toll” homology domain, which is necessary for subsequent signaling.

However, a chimeric receptor consisting of the complete IL-1RII plus the cytoplasmic part of IL-1RI is capable of signaling as shown by Heguy et al. (15). We used this chimeric receptor to generate a cell system in which signal transmission transited via IL-1RI could be measured.

Generation of permanent EL-4 clones expressing the chimeric IL-1R

The murine cell line EL4 D6/76 expresses about 2000 IL-1RI per cell but does not respond to IL-1 because it does not express IL-1RAcP. Transfection with IL-1RAcP reconstituted IL-1 responsiveness completely (9, 10), demonstrating that IL-1RI requires IL-1RAcP to transduce signals. To test whether the chimeric receptor would also need IL-1RAcP for signal transduction, we transfected EL-4 D6/76 with this construct. In parallel, we transfected the clone AcP1 which was derived from EL-4 D6/76 and overexpresses IL-1RAcP (10) with the chimeric receptor, so the resulting transfecants only differed in IL-1RAcP expression. Permanent clones were established from both pools. The expression of mRNA for the chimeric receptor and IL-1RAcP was ascertained by RT-PCR analysis (data not shown). The IL-1RAcP negative clone was termed D-Chim, the IL-RAcP positive clone Chim9.

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The chimeric IL-1R signals only in the presence of IL-1RAcP

IL-1 induces IL-2 synthesis in EL-4 cells (22). Expression of the chimeric receptor in the absence of IL-1RAcP by itself did not confer IL-1 responsiveness. Thus, D-Chim cells did not respond to IL-1 (Fig. 1A). In AcP1 cells and Chim9 cells IL-1 induced IL-2 production (Fig. 1A). As Chim9 cells express both the endogenous murine IL-1RI and the chimeric receptor, we had to demonstrate the contribution of the chimeric receptor to IL-1 signaling. Therefore, the endogenous mIL-1RI were blocked by increasing concentrations of the anti-mIL-1RI mAb 35F5, and the cells were stimulated with a constant concentration of 100 pg/ml rhIL-1α. Complete inhibition of IL-1 induced IL-2 synthesis was achieved in AcP1 cells that only posses mIL-1RI (Fig. 1A), whereas in Chim9 cells only a partial neutralization was observed even at high concentrations of blocking Ab. IL-1-mediated IL-2 production in Chim9 cells was completely inhibited by adding a blocking anti-hIL-1RII mAb to the system in which mIL-1RI had already been blocked by 100 ng/ml of the blocking anti-mAb 35F5 which neutralized signaling via the endogenous IL-1RI. The means ± SD of triplicates from one representative experiment is shown out of three with identical results.

The chimeric IL-1R signals only in the presence of IL-1RAcP

IL-1Ra shows a strong preference for IL-1RI (summarized in Ref. 23). IL-1Ra inhibited IL-1-induced IL-2 production in AcP1 cells with an ED_{50} of about 10 ng/ml (Fig. 2). In Chim9 cells, which express the chimeric receptor with the hIL-1RII binding site in addition to endogenous mIL-1RI, about 100-fold higher concentration of IL-1Ra was required to achieve a comparable degree of inhibition. This is in good accord with the published differences in affinities of IL-1Ra for IL-1RI and IL-1RII, respectively (23) and reflects the contribution of the chimeric receptor to IL-1 signaling in Chim9 cells.

Demonstration of the molecular interaction of IL-1RII and IL-1RAcP

To demonstrate direct protein-protein interaction of IL-1RII with IL-1RAcP, we cotransfected these molecules transiently into EL-4 D6/76 cells that express endogenous mIL-1RI, but not IL-1RII or IL-1RAcP. The transfectants were incubated with [^{125}I]IL-1α, and surface molecules were crosslinked with the homobifunctional crosslinker BS\textsuperscript{3}. Immunoprecipitates of mIL-1RI from mock-transfected EL-4 D6/76 cells showed a band at about 80 kDa which corresponds to the 80-kDa mIL-1RI with the 17-kDa [^{125}I]IL-1α covalently crosslinked to it (Fig. 3A, lane 1). When EL-4 D6/76 cells were transfected with IL-1RAcP, an additional complex of 180–200 kDa was immunoprecipitated that consists of mIL-1RI plus [^{125}I]IL-1α and IL-1RacP (Fig. 3A, lane 2). Due to the inefficient crosslinking reaction [^{125}I]IL-1α is partially liberated from the noncovalently associated complexes after heating the samples to 95°C. Therefore, free [^{125}I]IL-1α is detected migrating at 17 kDa.

To avoid interference of bands derived from IL-1RII complexes in the crosslinking experiments, we blocked mIL-1RI with mAb

FIGURE 1. Inhibition of IL-1 signaling mediated by IL-1RI. A, AcP1 cells (●, IL-1RAcP pos.), Chim9 cells (■, IL-1RAcP pos., IL-1RII/IL-1RI pos.), and D-Chim cells (▲, IL-1RAcP neg., IL-1RII/IL-1RI pos.) were treated with 50 pg/ml rhIL-1α and increasing concentrations of the blocking anti-mIL-1RI mAb 35F5 for 18 h. IL-2 was measured in the supernatants by ELISA. B, Inhibition of IL-1 signaling mediated by the chimeric IL-1RII/IL-1RI. Chim9 cells (■, IL-1RAcP pos., IL-1RII/IL-1RI pos.) were stimulated with 50 pg/ml rhIL-1α and increasing concentrations of a blocking anti-hIL-1RII mAb for 18 h in the absence (○) or in the presence (◼) of 100 ng/ml of the blocking anti-mAb 35F5 which neutralized signaling via the endogenous IL-1RI. The means ± SD of triplicates from one representative experiment is shown out of three with identical results.

FIGURE 2. Inhibition of IL-1 response by IL-1R antagonist reveals participation of the chimeric receptor in Chim9 cells. AcP1 cells (open bar, ●) and Chim9 (filled bar, ■) cells were treated with 100 pg/ml rhIL-1α and increasing concentrations of rhIL-1Ra for 18 h. IL-2 was determined in the supernatant by ELISA. One representative experiment is shown from three with identical results.
35F5 before adding [125I]IL-1α to the cells. Under these conditions no signal can be seen in EL-4 D6/76 cells, because no IL-1 binding sites are available (Fig. 3, lane 3). The same is true for cells only transfected with IL-1RAcP (Fig. 3B, lane 5) supporting the findings of Greenfelder et al. (8) that IL-1RAcP does not bind IL-1 itself. Cells transfected with hIL-1RII showed a faint band at about 80 kDa (Fig. 3B, lane 4). An additional band in the high m.w. range was always visible. Both complexes could be immunoprecipitated with an anti hIL-1RII mAb (data not shown), suggesting that they consist of [125I]IL-1α crosslinked to IL-1RII. This high m.w. complex may be a dimer of human IL-1RII, because such a comparable band was also observed in the human B-cell line RAJI, which expresses hIL-1RII but not IL-1RAcP or IL-1RI (D.L. and comparable band was also observed in the human B-cell line RAJI, m.w. complex may be a dimer of human IL-1RII, because such a combination of both (lane 6) and cultured for 24 h. After 24 h 100 ng/ml of blocking anti mIL-1RI mAb 35F5 were added before cells were incubated with [125I]IL-1α and crosslinked with BS3. Proteins from postnuclear lysates were separated by SDS-PAGE visualized by Phospholmagr. Data are from a single representative experiment of a series of five with comparable results.

Identification of IL-1RAcP and IL-1RII in the high m.w. complex contains IL-1RII and IL-1RAcP, both crosslinked to [125I]IL-1α.

The complex of IL-1RII and IL-1RAcP can be detected in normal human B cells

Crosslinking experiments were performed with normal human B cells in the presence of radiolabeled IL-1α. Three bands were observed in lysates from these B cells. Free [125I]IL-1α at 17 kDa, ligated hIL-1RII at 80 kDa, and a high m.w. complex of 180–200 kDa (Fig. 5, lane 1). A specific antiserum against hIL-1RAcP precipitated this high m.w. complex besides of the 82-kDa band (Fig. 5, lane 2), while a rabbit pre-immune serum yielded no bands (Fig. 5, lane 3). The residual lysate was specifically depleted of the high m.w. band (Fig. 5, lane 4) after immunoprecipitation of hIL-1RAcP. The same pattern was observed in B cells from four other donors (data not shown). These results show that the formation of a trimeric complex consisting of IL-1 bound to IL-1RII and IL-1RAcP is possible in normal human B cells.

Discussion

IL-1 binds to two types of specific plasma membrane receptors with different biological consequences for the target cell. The binding of IL-1 to IL-1RI results in a conformational change of the receptor which presumably allows the association of IL-1RAcP. The formation of this heterotrimeric complex of IL-1RII plus IL-1 and coreceptor is the prerequisite for subsequent intracellular signaling events. The binding of IL-1 to IL-1RII, however, does not initiate IL-1 signaling because IL-1RII lacks the cytoplasmic domain which is required to recruit appropriate adapter molecules. Still, the high degree of homology shared between IL-1RI and IL-1RII strongly suggests that both ligated receptors have similar three dimensional structures. In consequence, ligated IL-1RII would also interact with IL-1RAcP and form a heterotrimeric complex without transducing signals.

By employing chimeric receptors encompassing the extracellular and transmembrane portion of IL-1RII and the cytoplasmic domain of IL-1RI, we demonstrated that such an interaction takes place. Expression of chimeric receptors alone did not confer IL-1 responsiveness to cells lacking IL-1RAcP: Both endogenous mIL-1RI and chimeric hIL-1RI required IL-1RAcP to form a signaling complex. We demonstrated that both the natural and the chimeric receptor contributed to the IL-1 response in our cloned cells by sequentially blocking endogenous mIL-1RI and then hIL-1RII.

FIGURE 3. Demonstration of the physical association of IL-1RII and IL-1RAcP in the presence of IL-1α by crosslinking experiments. A. Demonstration of mIL-1RI and IL-1RII/IL-1RAcP complexes. EL-4 D6/76 cells were left untreated (lane 1) or transiently transfected with murine FLAG-tagged IL-1RAcP (lane 2) cultured for 24 h. [125I]IL-1α was added to intact cells and crosslinked using BS3. Cells were washed and lysed, and nuclear debris were removed by centrifugation. mIL-1RI was immunoprecipitated with the mAb 12A6 which recognizes ligated mIL-1RI. Proteins from these precipitations were separated by SDS-PAGE, and IL-1 binding proteins were visualized with the Phospholmagr. B. Crosslinking with [125I]IL-1α after blocking the endogenous mIL-1RII. EL-4 D6/76 cells were left untreated (lane 3) or transiently transfected with either human IL-1 RI (lane 4), murine FLAG-tagged IL-1RAcP (lane 5), or a combination of both (lane 6) and cultured for 24 h. After 24 h 100 ng/ml of blocking anti mIL-1RI mAb 35F5 were added before cells were incubated with [125I]IL-1α and crosslinked with BS3. Proteins from postnuclear lysates were separated by SDS-PAGE visualized by Phospholmagr. Data are from a single representative experiment of a series of five with comparable results.
assumed that heterodimerization is mediated by the extracellular recognition of the ligated receptors by IL-1RcP. Indeed, recently Huang et al. (24) showed that the cytoplasmic tails are not required for IL-1-mediated interaction of the extracellular domains of IL-1RI and IL-1RcP.

Initiation of signal transduction by IL-1, however, quite clearly requires the cytoplasmic part of IL-1RI (25–27) and very recent results suggest that also the cytoplasmic part of IL-1RcP is indispensable for efficient signaling to occur (11, 12, 24, 28). Thus, one could argue that the cytoplasmic tails of IL-1RI and IL-1RcP may contribute to the formation of the heterotrimeric complexes, an interaction which would be possible in IL-1RI and the chimeric receptor but obviously not in the natural IL-1RI.

We showed the direct protein-protein interaction of normal human IL-1RII with IL-1RcP by crosslinking these two molecules in the presence of radiolabeled IL-1. A high m.w. complex was identified which consisted of IL-1 bound to hIL-1RII and the coreceptor molecule as demonstrated by consecutive immunoprecipitations with the respective Abs. This result shows that the cytoplasmic tail of the chimeric receptor is not required for ligand mediated association in our clones. Comparison of the heterotrimeric complex containing either hIL-1RII or mIL-1RI showed no detectable difference in the apparent m.w. determined in the gels, although the two ligated receptor types showed a clear difference of 15–17 kDa. This may be due to sterical effects in the crosslinked components affecting the electrophoretic mobility. In addition, the band of the high m.w. complex was relatively diffuse, probably due to the fact that IL-1Rs and IL-1RcP are heavily glycosylated. The size of the heterotrimeric complexes we observed in our transfected cells is in good accordance with the complex of IL-1RI plus IL-1 and IL-1RcP reported by Greenfeder et al. (8).

Finally, we investigated whether such a heterotrimeric complex of hIL-1RII plus IL-1 and hIL-1RcP could be visualized in normal cells. And indeed, we were able to demonstrate a high m.w. complex in normal human B cells that predominantly express IL-1RII. hIL-1RcP was identified as component of this high m.w. complex in human B cells by immunoprecipitation with a specific antiserum.

In summary, the data presented here show that IL-1RII and IL-1RcP can interact in the presence of IL-1. This may have important biological consequences. We propose that this interaction will result in a competition of IL-1Rs for the coreceptor. And as IL-1RcP is indispensable for signaling via IL-1RI, the interaction...
with IL-1RII should have an effect on IL-1 responsiveness. Indeed, if IL-1RII is expressed at a much higher frequency than IL-1RI this leads to a decrease in sensitivity to IL-1 as observed in fibroblasts (17) and keratinocytes transfected with hIL-1RII (29). This result was explained by the authors as capture and sequestration of ligand by IL-1RII, the so-called ligand sink effect. In addition to this effect, we propose a novel regulatory role for IL-1RII based on the observation that ligand-bound IL-1RII can sequester IL-1RAcP into a nonfunctional complex. IL-1RII can compete for the coreceptor and distract IL-1RAcP from the signaling complex with IL-1RI. A model of this coreceptor competition is depicted in Fig. 6. In this model the ratio of IL-1RI to IL-1RII defines the threshold at which a given cell can respond to IL-1, if the availability of IL-1RAcP is limited. In this view, regulation of surface expression of IL-1RAcP becomes of pivotal importance in determining responsiveness to IL-1. Presently, little data are available, and they show that only few accessory molecules are expressed per cell (International Patent Application Number WO 96/23067 by Hoffmann-La Roche AG, Basle, Switzerland). In addition, IL-1RAcP does not seem to be significantly regulated at the mRNA level (D.L. and M.U.M., unpublished data) while surface expression of IL-1RAcP is tightly regulated. On one hand anti-inflammatory cytokines and drugs, such as IL-4 and glucocorticoids, can up-regulate IL-1RII by stimulating de novo synthesis (7). On the other hand, IL-1RII can be shedded from the surface rapidly by proteolytic cleavage (30), a process which is highly regulated by TNF and IL-8 (31).

We thus propose that the role of IL-1RII in the tightly regulated IL-1 system is far more sophisticated than that of a pure scavenger of IL-1. IL-1RII can subtract IL-1RAcP from the IL-1RII signaling complex. The ratio of IL-1RII to IL-1RI can attenuate the cell threshold of IL-1 responsiveness. In the course of inflammation, a given cell type could change this ratio in both directions, by shedding of membrane IL-1RII or by de novo synthesis of IL-1RII depending on the cell type, environmental conditions, and stage of inflammation. The competition of the decoy IL-1RII for the coreceptor is a novel mechanism of regulating cytokine responsiveness. Its uniqueness to the IL-1 system emphasizes the importance nature has given to the regulation of IL-1 in inflammation.

Note. While this manuscript was in review, Malinowsky et al. (32) reported on the interaction of IL-1RAcP with IL-1RII in transfected HEK-293 cells using rhIL-1β.

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