The Interleukin-1 Receptor Accessory Protein (IL-1RAcP)
Is Essential for IL-1-induced Activation of Interleukin-1 Receptor-associated Kinase (IRAK) and Stress-activated Protein Kinases (SAP Kinases)*

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The Interleukin-1 (IL-1) is a central mediator of the immune system involved in acute and chronic inflammatory responses. Although the sequences of two types of IL-1 receptors are known, the exact molecular events resulting in signal transduction and coupling to downstream signaling elements remain unclear. The recently cloned IL-1 receptor accessory protein (IL-1RAcP) has been suggested as a co-receptor molecule for IL-1RI, supported by the observation that its expression correlates to IL-1 responsiveness. We transfected the EL-4 subline D6/76 with IL-1RAcP cDNA. This cell line is an IL-1 non-responder expressing IL-1RI but lacking constitutive IL-1RAcP expression. The expression of IL-1RAcP in EL-4 D6/76 was sufficient to restore IL-1-induced activation of interleukin-1 receptor-associated kinase and of stress-activated protein kinases, translocation of the transcription factors NFκB and IL-1 NF to the nucleus, and induction of IL-2 mRNA synthesis. These results proved that IL-1RAcP is an indispensable molecule in the IL-1 receptor signal transduction complex, necessary to link events on the plasma membrane level to downstream signaling pathways, allowing IL-1-dependent activation of transcription factors and gene expression.

IL-11 is a pro-inflammatory cytokine centrally involved in regulating local and systemic responses of the immune system. It exerts its many biological effects on a wide variety of target cells through specific plasma membrane receptors (1). Two types of IL-1 receptors have been cloned so far (2, 3), of which only the type I IL-1 receptor (IL-1RI) initiates signal transduction (4), whereas the type II IL-1 receptor (IL-1RII) presumably functions solely as a ligand sink, as a decoy receptor (5). Although it is clear that the cytoplasmic tail of IL-1RI is necessary to initiate cytoplasmic signaling (6), the molecules involved and the mechanisms utilized remain obscure. The recent identification of a second subunit to the IL-1RI, the IL-1 receptor accessory protein (IL-1RAcP) (7) places the IL-1 receptor into the group of multimeric cytokine receptors. Homology searches and sequence comparisons show a protein kinase C docking site and a putative GTPase domain in IL-1RAcP, but it remains to be shown that these play any role in the biological function of this molecule. Recently we found that IL-1RAcP mRNA is constitutively expressed in a wide variety of cell types and that its expression correlates with IL-1 responsiveness. Cells lacking IL-1RAcP did not respond to IL-1 (8), suggesting that the signal transduction machinery initiating IL-1 signaling is dependent on the presence of IL-1RAcP.

Here we show that the IL-1RAcP is indispensable in linking the first step in IL-1 signal generation, namely the binding of IL-1 to IL-1RI, to two important elements of downstream signaling, the IL-1RI-associated protein kinase IRAK and the stress-activated protein kinases (SAP kinases). This results in activation and translocation of transcription factors as well as in IL-2 gene transcription and IL-2 production. We employed a subclone of the murine thymoma cell line EL-4 which expresses functional IL-1RI molecules and binds IL-1, but lacks mRNA for the IL-1RAcP. By transfecting this cell line with IL-1RAcP cDNA we established permanent cell lines expressing IL-1RAcP, which were all responsive to IL-1. Thus IL-1-induced activation of IRAK and SAP kinases, IL-1 NF and NFκB translocation, and IL-2 mRNA synthesis were restored.

These results demonstrate that IL-1RAcP is an absolutely necessary constituent of the multimeric IL-1 type I receptor signal transduction complex in the plasma membrane, guaranteeing the coupling to such important downstream signaling elements as IRAK and the SAP kinases.

EXPERIMENTAL PROCEDURES

Plasmids and Mammalian Cell Culture—The expression plasmid pEF-AcP was engineered using PCR cloning techniques. With a 5'-primer from position 131 to 148 and a 3'-primer from 1841 to 1860 of the published sequence of mIL-1RAcP (7) a PCR was performed with cDNA from the murine thymoma cell line EL-4. The PCR product was introduced into the pCRII vector (Invitrogen) via TA cloning, cleaved with EcoRI, blunted, ligated to Xba linkers, and introduced into the Xba site of pEF-BOS (9). The sequence of the insert was checked by sequencing.

Mammalian cell culture was carried out at 37 °C, 5% CO2. The cells were cultured in RPMI 1640 (Life Technologies, Inc.) supplemented with 5% (v/v) fetal calf serum, 1 mM pyruvate (Life Technologies, Inc.), non-essential amino acids (minimum essential medium, Life Technologies, Inc.), and 2 mM L-glutamine (Life Technologies, Inc.). The cells were passaged every 2–3 days to maintain a cell density of 10^5–10^6 cells/ml.

Generation of Stable Cell Lines—EL-4 D6/76 cells were transfected
with pcDNA III (Invitrogen) in combination with pEF-AcP or pEF-BOS (vector control) by electroporation. Cells were washed with cold PBS, and 107 cells were suspended in 0.5 mL of PBS and incubated with or without 20 μg of pcDNA3 and 150 μg of pEF-AcP or pEF-BOS for 10 min on ice. Electroporation was performed at 200 V with a capacity of 1000 microfarads. Cells were left on ice for 10 min and were finally resuspended in 10 mL of medium, supplemented additionally with penicillin/streptomycin (Life Technologies, Inc.). After 24 h cells were centrifuged and resuspended in 10 mL of medium with penicillin/streptomycin and 600 μg/mL G418 (Boehringer Mannheim). After 2 weeks of cell culture G418-resistant cell pools were obtained (vector control, EL-4 D6/76 BOS, EL-4 1R AcP, EL-4 D6/76 AcP). Single cell clones of the AcP transfected were isolated by limiting dilution, screened by RT-PCR for IL-1R expression and IL-1 responsiveness, and named EL-4 AcP. Several rounds of electroporation and subcloning were performed. Six subclones were analyzed in detail termed EL-4 AcP1, -2, -5, -6, -7, and -8.

Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)—RT-PCRs were performed as described previously (8) with slight modifications. For measurement of IL-1-induced IL-2 mRNA 5 × 106 cells were incubated in 10 mL of medium for 6 h with or without 10 ng/mL IL-1β (5–10 × 104 units/mg, Schlo, Siena, Italy). RNA was extracted using the RNeasy Kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions followed by a DNase I digestion. The PCR was performed with an annealing temperature of 65 °C for 35 cycles with the following primers (synthesized by Pharmacia, Freiburg, Germany). The results were standardized via the amplification product of aldolase.

IL-1R AcP (product length 677 base pairs): primer 1, 5'-AAC CAT CGG TCA CTT GGT ATA AGG G-3'; primer 2, 5'- TTC ATC TGT TCC AAA GTG AGC TCG G-3'. IL-2 (product length 412 base pairs): primer 1, 5'-AAG CTC CAC TTC AAG CTC TAC AGC G-3'; primer 2, 5'-TTG ACA GAG GCC TAT CCA TCT CCT C-3'.

Northern Blot Analysis—RNA was prepared as described above. 20 μg of total RNA per lane were separated on a 1.2% agarose/formaldehyde gel and blotted on a Nylon membrane (Qiagen, Hilden, Germany) according to standard procedures using 20 × SSC (3 M NaCl, 3.3 M sodium citrate) (14). The RNA was cross-linked by UV irradiation and subsequently hybridized with cDNA probes specific for murine IL-1RAcP, IL-1RI, or murine GAPDH. The probes were labeled with [32P]dCTP (111 TBq/mmol, Hartmann, Braunschweig, Germany) using the diprime DNA labeling system (Amersham, Braunschweig, Germany) according to the manufacturer’s instructions. Hybridization was performed in the presence of dextran sulfate and formamide at 42 °C for 20 h. The membranes were washed twice with 6 × SSC, 0.1% SDS at 42 °C for 30 min. A second hybridization was performed with 3 × SSC, 0.1% SDS at 60 °C and 30 min in 1 × SSC, 0.1% SDS at 60 °C. The signals were visualized by autoradiography, quantitated by phosphoimaging (Model GS-250, Bio-Rad, Munich, Germany), and standardized via the GAPDH signal. Prior to the next hybridization the blots were stripped by incubation in boiling 2 × SSC, 0.1% SDS for several seconds followed by slowly cooling to room temperature.

SAP Kinase Activation—SAP kinase activation was measured as described recently (10). In brief, 2 × 105 cells/sample were incubated with or without 10 ng/mL IL-1β. Cells were washed, lysed, and immunoprecipitated with an anti-IL-1RI antibody (12A6, Pharmingen), and the immunoprecipitates were subjected to an in vitro kinase assay with radiolabeled ATP. The phosphorylated proteins were separated by SDS-PAGE and visualized by autoradiography.

SAP Kinase Inhibition—SAP kinase activation was determined as described elsewhere (11). 5 × 105 cells were incubated in 1 mL of medium with or without 10 ng/mL IL-1α (10 × 104 units/mg, Immunex, Seattle, WA), 10 ng/mL IL-1β, or 500 mM sorbitol (Sigma) for 15 min. Cells were washed with ice-cold PBS, resuspended in lysis buffer (20 mM Tris, pH 7.4, 1 mM EDTA, 1 mM EGTA, 2 mM DTT, 0.5% Triton), and incubated for 30 min on ice. After centrifugation (30 min, 13,000 × g, 4 °C) the protein concentrations of the supernatants were determined using the Bradford assay (Bio-Rad). SAPK activity in these supernatants was measured in the Bradford assay (Bio-Rad). SAPK activity in these supernatants was measured in the Bradford assay (Bio-Rad).

AcP Kinase Activity—AcP kinase activity was determined as described elsewhere (11). 5 × 105 cells were incubated in 1 mL of medium with or without 10 ng/mL IL-1α (10 × 104 units/mg, Immunex, Seattle, WA), 10 ng/mL IL-1β, or 500 mM sorbitol (Sigma) for 15 min. Cells were washed with ice-cold PBS, resuspended in lysis buffer (20 mM Tris, pH 7.4, 1 mM EDTA, 1 mM EGTA, 2 mM DTT, 0.5% Triton), and incubated for 30 min on ice. After centrifugation (30 min, 13,000 × g, 4 °C) the protein concentrations of the supernatants were determined using the Bradford assay (Bio-Rad). SAPK activity in these supernatants was measured as described above.

Electrophoretic Mobility Shift Assays—For the preparation of nuclear extracts 2 × 105 cells in a density of 4 × 107 cells/mL were incubated with or without 10 ng/mL IL-1α or IL-1β for 1 h at 37 °C. centrifuged (1,200 rpm, 8 min), washed twice with ice-cold PBS, and incubated for 15 min on ice in buffer A (10 mM HEPES, 10 mM KCl, 100 mM EDTA, 100 μM EGTA, 1 mM DTT, 500 μM phenylmethylsulfonfyl fluoride). After adding 25 μL of a 10% Nonidet P-40 solution, the mixture was centrifuged for 30 s at full speed in a tabletop centrifuge. The supernatant was immediately removed, and the pellet was resuspended in 50 μL of buffer C (20 mM HEPES, 400 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, 1 mM phenylmethylsulfonfyl fluoride), incubated for 15 min on ice, and centrifuged (5 min, 13,000 × g, 4 °C). The protein concentration of the supernatants was measured with a Bradford assay.

As probe double-stranded oligonucleotides with the sequence TGA CAG AGG CTA TCC AGA GA (for NFκB, derived from Ref. 12, synthesized by Pharmacia Biotech Inc.) or GAT CAC CAA GGA TTT CAC CTA AAT CC (for IL-1 NF, derived from Ref. 13) were radioactively labeled with the T4 polynucleotide kinase using standard procedures (14). For the binding reaction 1 μL of nuclear extract (5 μg/μL) was incubated with 1 μL of the labeled oligonucleotide (−0.1 pmol with 100,000 rpm), 1 μL of competitor DNA (2 μg/μL poly(dI-dC)), and 1 μL of HEP buffer (10 mM Tris, 10 mM EDTA, 10 mM DTT, 500 mM NaCl, 50% glycerol, pH 7.5) for 30 min at room temperature. The protein/nucleotide complexes were separated in a 4% polyacrylamide gel. Electrophoresis was performed for 0.25 h at TBE at 10 V/cm. For visualization of the labeled oligonucleotide the dried gel was exposed to a Biomax MR x-ray film (Kodak) overnight at −80 °C.

Quantitation of IL-2 Production—Cells of the different clones were seeded at a density of 5 × 104 cells/well in 96-well flat bottom microtiter plates (Falcon 1640 plus 10% fetal calf serum and the calcium ionophore A23187 (Sigma, 2.5 × 10−7 M)). IL-1 was added at 10, 100, and 1000 pg/mL final concentration (rhIL-1α or rhIL-1β). Cells were incubated for 24 h, and then supernatants were removed. IL-1 was measured using the Genzyme DuoSet for mouse IL-2 according to the manufacturer’s instructions (Genzyme, Cambridge, MA).

RESULTS

EL-4 D6/76 Lack IL-1R AcP: Transfection with IL-1R AcP—EL-4 D6/76 (D6/76) cells were identified as IL-1 non-responders by von Hoegen et al. (15). This subline of the murine thymocyte cell line EL-4 expresses several thousand 90-kDa type 1 IL-1 receptors per cell and binds IL-1α and IL-1β with an affinity comparable to the IL-1 responder EL-4 6.1 (Ref. 15 and own results, not shown). By RT-PCR analysis we could not detect mRNA coding for transmembrane murine IL-1R AcP. We assumed that the lack of IL-1R AcP might be the reason for the cell’s unresponsiveness to IL-1. Therefore we transfected D6/76 cells with a vector containing the full coding sequence of the murine transmembrane IL-1R AcP. By limiting dilution we established several permanent cell lines which expressed IL-1R AcP mRNA and responded to IL-1. Here we show the results obtained for one clone, EL-4 AcP1, and, as a control, a cell line transfected with the empty vector (EL-4 D6/76 BOS = D6/76 BOS) (Fig. 1). The other IL-1R AcP-positive clones responded in...
IL-1RAcP and IL-1 Signal Transduction

**FIG. 2. Expression of IL-1RAcP is required for IRAK activation.** Cells were stimulated for 10 min with or without 10 ng/ml IL-1. An *in vitro* kinase assay was performed with the IL-1RI immunoprecipitates of different cell lines. The phosphorylation of exogenous (MBP and H1) and endogenous substrates (p60) was investigated upon IL-1 stimulation.

**FIG. 3. SAP kinase activity can only be induced by IL-1 in cells expressing the IL-1RAcP.** The four investigated cell lines were stimulated for 15 min with or without 10 ng/ml IL-1 or 500 mM sorbitol (*Stimulus*: −, control; I, IL-1; S, sorbitol). Crude cytosolic extracts were analyzed in an *in vitro* kinase assay with the specific substrate GST-c-Jun(1–79).

**FIG. 4. IL-1 RAcP expression restores IL-1-induced NFkB (A) and IL-1 NF activation (B).** Nuclear extracts of cells stimulated for 1 h with or without 10 ng/ml IL-1 were incubated with radioactively labeled oligonucleotides coding for the NFkB (A) or IL-1 NF (B) binding sites for 30 min, and the resulting protein-DNA complexes were separated by PAGE.

**Transcription Factors Are Activated by IL-1 Signaling Pathways Only in IL-1RAcP-expressing EL-4 Cells—**IRAK can couple to NFkB utilizing the TRAF6 molecule (18), whereas c-Jun kinases can activate Ets proteins like Elk-1 or possibly c-Jun directly. IL-1 induced the activation and nuclear translocation of NFkB and IL-1 NF only in IL-1RAcP-positive cell lines, whereas no shift could be observed in electrophoretic mobility shift assays performed with nuclear proteins extracted from IL-1-stimulated EL-4 D6/76 or D6/76 BOS cells (Fig. 4). An IL-1-induced AP-1 or Elk-1 shift could not be detected in EL-4 unambiguously (data not shown).

**IL-1 Activation of EL-4 Results in IL-2 mRNA Synthesis if IL-1RAcP Is Expressed—**The murine T cell line EL-4 produces the lymphokine IL-2 after IL-1 treatment (19). A constitutive, but very low, expression of IL-2 mRNA could be detected by RT-PCR in all EL-4 lines. IL-1 stimulation of EL-4 6.1 and IL-1RAcP-expressing clones resulted in a marked enhancement of IL-2 mRNA synthesis, while no change of IL-2 mRNA expression could be observed in the cell lines lacking IL-1RAcP (data not shown).

**Analysis of IL-1RAcP mRNA Expression and Correlation with IL-1 Response—**Six individual clones expressing IL-1RAcP after transfection were analyzed in detail by Northern blot analyses to quantitate levels of mRNA for IL-1RAcP in comparison to EL-4 6.1, EL-4 D6/76, and D6/76 BOS cells. Five out of six transfectants expressed relatively high levels of IL-1RAcP mRNA in comparison to EL-4 6.1, whereas EL-4 AcP2 showed only a modest expression even lower than EL-4 6.1. EL-4 D6/76 and D6/76 BOS cells expressed no mRNA for IL-1RAcP (Fig. 5A), the smaller size of the IL-1RAcP mRNA in the
transfectants is due to the lack of the 5'- and 3'-untranslated regions). All of the transfectants responded to IL-1 by producing IL-2 in a concentration-dependent manner, whereas EL-4 D676 and D676 BOS cells did not produce detectable amounts of IL-2 (Fig. 6). The transfectant EL-4 AcP/2 which expressed the lowest level of IL-1RAcP mRNA responded to IL-1 in a comparable dose-response curve to EL-4 6.1, reaching roughly the same level of IL-2 production in the 24 h observed. The transfectants expressing high levels of IL-1RAcP mRNA responded to IL-1 in a comparable dose range but did not reach the same level of IL-2 production. In all cell lines mRNA for IL-1RI was expressed in comparable amounts with the exception of EL-4 AcP/6 and EL-4 AcP/8 which showed about one-fourth the intensities of EL-4 D676 (Fig. 5B).

**DISCUSSION**

The aim of this study was to clarify the role of IL-1RAcP in IL-1RI-induced signal transduction. We observed that in the absence of IL-1RAcP no response could be detected to IL-1 in EL-4 D676 and other cell lines (8). We transfected the IL-1 non-responder cell line EL-4 D676 with IL-1RAcP and investigated whether IL-1 responsiveness could be restored. We generated several permanent IL-1RAcP-positive subclones and analyzed signaling events reported to occur after IL-1 binding to its receptor at different levels in the cell, including very early events, cytokine kinase cascades, the activation and nuclear translocation of transcription factors, gene transcription, and finally IL-2 production.

IL-1 binding and induction of signal transduction is probably complex. Presently, the following model emerges: once IL-1 has bound to its receptor it is highly likely that a complex of ligand, IL-1RI, and IL-1RAcP is formed on the outside of the cells, which results in the close association of the cytoplasmic tails of the IL-1RI as well as the IL-1RAcP. Presently it is not clear whether IL-1RAcP is constitutively located at the IL-1RI molecule or whether it associates with the IL-1RI after this has bound IL-1. The stoichiometry of the complex is also unknown, the minimal model being a heterodimer with the possibility of two or more of these heterodimers forming a complex.

The heterodimer (in the minimal model) may serve as a scaffold for the association of additional molecules which is involved in translating this heterodimerization into one or several cytoplasmic signals. When is well established that the cytoplasmic tail of IL-1RI is required for IL-1 signaling events to occur in the cytoplasm, and certain domains have been defined which have to be present to facilitate IRAK activation, for example (6, 16, 20). It is not known presently if the cytoplasmic tail of IL-1RAcP is needed for signaling or which parts may be dispensable.

The molecules directly involved in the initial process of signal generation remain to be identified, although some candidates have been published in the last years. We found a serine/threonine-specific protein kinase associated with the IL-1RI after IL-1 stimulation of cells (10). This molecule was termed IRAK (16) and has been cloned recently (17). It is co-precipitable with IL-1RI only in cells which have been activated by IL-1 treatment. We could not observe IRAK activation in cells lacking IL-1RAcP, but found this kinase active in IL-1RI immunoprecipitates from the transfectant cell lines.

Its activation in less than 30 s points to a high position in the hierarchy of IL-1-induced signaling events. In IL-1-activated cells TRAF6 was very recently identified as a molecule which is interacting with IRAK and which is involved in NFkB activation (18).

Novel members of the mitogen-activated protein kinases are activated by IL-1 (11, 21). These mitogen-activated protein kinases phosphorylate the N-terminal activation domain of c-Jun and can be activated by inflammatory cytokines such as IL-1 and tumor necrosis factor and by extracellular stress. Accordingly they have been termed stress-activated protein kinases (SAPKs) or c-Jun N-terminal kinases (JNKs). The link between these kinases and the IL-1 receptor complex has not yet been identified. They are activated by such diverse stimuli as cytokines, osmotic stress, or UV light, and a recent publication demonstrates that stress may lead to membrane perturbation resulting in an oligomerization of cytokine and growth factor receptors in the absence of specific ligands. This mechanism may result in the utilization of the respective signaling pathways leading to the activation of SAP kinases (22).

In all EL-4 cell types employed for the studies discussed here, stress, e.g. by hyperosmolality, potently activated stress kinases, which resulted in the phosphorylation of the exogenously added GST-c-Jun fusion protein. However, IL-1 was...
only able to induce GST-c-Jun phosphorylation if the cell lines expressed IL-1RACP, emphasizing the need for this molecule also for this branch of IL-1 signaling.

IRAK results indirectly in the activation of the transcription factor NFκB (18). The SAP kinases may directly phosphorylate transcription factors of the Ets family or c-Jun. We analyzed the activation of NFκB, IL-1 NF, AP1, and Ets-like proteins in IL-1RACP-positive and -negative EL-4 lines in nuclear extracts. IL-1 treatment of the cells resulted in the activation and nuclear translocation of the transcription factors only if IL-1RACP was expressed. In our systems we could not observe an IL-1-induced AP-1 shift, and the activation of Ets-like proteins was not significant; however, NFκB and IL-1 NF were dramatically activated after IL-1 stimulation. The participation of the IRAK/TRAF6 complex or the SAP kinase pathway(s) in NFκB activation is not fully understood, and possible cross-talk between these two groups of kinases remains to be established, but both pathways require the expression of IL-1RACP to function.

The contribution of IL-1RI and IL-1RACP to IL-1 binding seems rather clear. While IL-1RI recognizes and binds IL-1 with an affinity in the nanomolar range, IL-1RACP itself does not bind IL-1 but interacts with the ligated IL-1RI, thereby increasing the affinity of IL-1RI for IL-1 about 5-fold as reported by Greenfeder et al. (7). In CHO transfectants expressing only murine IL-1RI (700,000 receptors/cell) a KD of 1.2 nM was measured, while co-transfection of both murine IL-1RI and murine IL-1RACP in CHO cells (20,000 receptors/cell) resulted in a KD of 0.25 nM (7). In the EL-4 system we did not observe an increase in affinity after expressing IL-1RACP, and Scatchard analyses of IL-1RACP transfectants showed no significant differences in KD values of IL-1RACP-negative or -positive EL-4 clones (23). However, one has to keep in mind that already the comparison of the IL-1RACP-positive IL-1 responders EL-4 D6.1 or EL-4 5D3 with the IL-1RACP-negative IL-1 non-responder EL-4 D6/76 showed no difference in affinity, all clones having a KD of 0.6–1.2 nM (15, 23). This suggests that the EL-4 system may not be suitable for evaluation of changes of affinity.

All IL-1RACP-positive clones responded to as little as 10 pg/ml IL-1 (there was no difference between IL-1α or IL-1β) by producing IL-2 with a qualitatively comparable dose-response curve. This was not influenced by the level of IL-1RACP expression, suggesting that in the EL-4 system IL-1RACP has no pronounced influence on the affinity of IL-1RI for IL-1. These data imply that the sensitivity of EL-4 cells to respond to IL-1 which is an indirect reflection of affinity is determined by IL-1RI and that at least in EL-4 an additional increase in affinity is neither achieved by IL-1RACP expression nor necessary to elicit a biological response such as IL-2 production. Although EL-4 AcP6 and EL-4 AcP8 expressed only one-fourth of the level of mRNA for IL-1RI they showed the same dose-response curve like the other clones, suggesting that it is only necessary to occupy a small fraction of the type I IL-1 receptors available to achieve a full signal, an observation already made by Iwasaki et al. (12).

The detailed contribution of IL-1RI and IL-1RACP to IL-1 signaling is unclear. Our data show that IL-1RACP is required to activate intracellular IL-1 signaling pathways and only IL-1RACP-positive clones are able to respond to IL-1. Interestingly, the strength of the IL-1 response, i.e. the saturation level of the IL-2 produced by EL-4 cells, was inversely correlated with IL-1RACP mRNA expression. The clones expressing high levels of IL-1RACP mRNA (and presumably high levels or IL-1RACP protein as well) were sensitive responders to IL-1 but weak producers of IL-2, whereas the only clone expressing low levels of IL-1RACP mRNA, EL-4 AcP/2, reached the level of IL-2 produced by EL-4 6.1.

This result suggests that the intracellular part of IL-1RACP is involved in modulating the strength of the IL-1 signal. One possible way of explaining this observation is the following working model. In the normal situation only a few IL-1RI molecules have to bind IL-1 to allow interaction with a few IL-1RACP molecules which results in a full IL-1 response (12). It is conceivable that a signal-transducing or -coupling component of the receptor complex is associated with the intracellular part of IL-1RACP and introduced into the signaling complex by the heterodimerization induced by the recognition of ligated IL-1RI by IL-1RACP on the extracellular side. If this putative signaling or coupling component is available only in low copy numbers, overexpression of IL-1RACP mRNA leading to an excess of IL-1RACP molecules in the plasma membrane may result in the “dilution” of this essential signaling molecule, abrogating its adequate participation in IL-1 signaling.

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