Interleukin-1 receptor accessory protein interacts with the type II interleukin-1 receptor

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Received 24 March 1998

Abstract Stably transfected HEK-293 cells express on their surface the murine type II IL-1 receptor (mIL-1RII) as demonstrated by FACS analysis using the mAb 4E2, however binding of [^{125}I]-hrIL-1β to these cells is nearly absent. Saturable high affinity binding of [^{125}I]-hrIL-1β is observed when the murine IL-1 receptor accessory protein (mIL-1RAcP) is coexpressed with mIL-1RII. Binding of [^{125}I]-hrIL-1β to mIL-1RII-mIL-1RAcP complex can be inhibited either with antibodies to mIL-1RII (mAb 4E2), or by antibodies to mIL-1RAcP (mAb 4C5). The number of high affinity binding sites in cells stably transfected with the cDNA for mIL-1RII is dependent on the dose of cDNA for mIL-1RAcP used to transfect the cells. The high affinity complex between mIL-1RII and mIL-1RAcP is not preformed by interaction between the intracellular domains of these two transmembrane proteins, rather it appears to require the extracellular portions of mIL-1RII and mIL-1RAcP and the presence of a ligand. We suggest that in addition to its earlier described decoy receptor role, IL-1RII may modulate the responsiveness of cells to IL-1 by binding the IL-1RAcP in unproductive/non-signalling complexes and thus reducing the number of signalling IL-1RI-IL-1RAcP-agonist complexes when IL-1 is bound.

Key words: Interleukin-1; IL-1 receptor type II; IL-1 receptor accessory protein; Interaction

1. Introduction

The cytokine interleukin-1 (IL-1) is a mediator of immune and inflammatory responses (for review, see [1]). The 'interleukin-1 system' possesses three ligands, two receptor subtypes, and one receptor accessory protein, respectively. The IL-1 receptor agonists, IL-1α and IL-1β, have overlapping biological activities, which can be blocked by excess of the endogenously occurring IL-1 receptor antagonist (IL-1ra) [2]. Two IL-1 receptor subtypes have been cloned from mammalian cells: the 80 kDa type I receptor (IL-1RI), and the 68 kDa type II receptor (IL-1RII) [3,4]. The discovery of the murine IL-1 receptor accessory protein (mIL-1RAcP) as a part of a signalling IL-1 receptor-ligand complex was long awaited as most functional cytokine receptors form oligomeric complexes [5]. It has been shown that the IL-1RI can form a complex with IL-1RAcP in the presence of agonists but not in the presence of the antagonist [5]. The IL-1RI and the IL-1RAcP have both been shown to be essential for IL-1 mediated signalling in vivo and in vitro [6–9]. However, there is a finding also suggesting that there is IL-1-IL-1RI induced neutral sphingomyelinase activity that is independent of the IL-1RAcP [8]. In binding assays the IL-1RII has been shown to act as an high affinity IL-1 binding protein, without any subsequent intracellular signalling to occur [10]. IL-1RII is shedded from the cell upon IL-1 stimulation [11] and an alternatively processed IL-1RII has also been described [12], providing in addition to the membrane bound, non-signalling IL-1RII, a soluble decoy target for IL-1 agonists. Until now, no information on an interaction of IL-1RAcP with IL-1RII has been presented.

Here we describe that the membrane bound mIL-1RII has an absolute requirement for the presence of mIL-1RAcP, for the high affinity binding of hrIL-1β. In addition we present data suggesting that the interaction between mIL-1RII and mIL-1RAcP is not involving the intracellular portions of these proteins.

2. Materials and methods

2.1. Eukaryotic expression constructs

The cDNA encoding the murine IL-1RII was subcloned from pRep3/mIL-1RII (kindly provided by Dr. J.E. Sims) into the pcDNAI vector (Invitrogen). The pcDNAI/mIL-1RAcP, for expression of the mIL-1RAcP, was obtained by RT-PCR from total RNA isolated from the murine hypothalamic GT1-7 cell line. The resultant cDNA fragment was cloned, sequenced, and digested with restriction enzymes and shown to yield the expected restriction fragment sizes.

2.2. Cell culture and transfection

Human embryonic kidney fibroblast cell line (HEK-293, ATCC) was grown in MEM supplemented with 10% (v/v) foetal bovine serum, L-glutamine, 50 μg/ml gentamycin, 100 μg/ml penicillin/streptomycin. For transfection experiments cells were grown in 100 mm cell culture dishes until 60% confluency. Cells were transfected by a Ca-phosphate method using 20 μg of plasmid as described [13], and harvested within 24–48 h. To generate HEK-293 cells stably expressing the mIL-1RII, transiently transfected cells were transferred to medium supplemented with 300 μg/ml geneticin (G418, Life Technologies) and allowed to grow an additional two weeks before clones were isolated.

2.3. [^{125}I]-hrIL-1β binding to whole cells

Transfected HEK-293 cells were harvested and washed once in binding buffer (RPMI 1640; 25 mM HEPES, pH 7.2; 1% BSA (w/v); 0.1% NaN_3 (w/v)). The cells were incubated with 50 pM [^{125}I]-hrIL-1β (specific activity 150 μCi/μg, DuPont, NEN, Boston, MA) in the absence or presence of 100 nM unlabelled hrIL-1β as competitor. To demonstrate selective expression of receptor subtypes blocking mAb 4C5, anti-mIL-1RAcP 10 μg/ml and blocking mAb 4E2, anti-mIL-1RII 3.5 μg/ml (kindly provided by Dr. R. Chizzonite) were used. Displacement curves were generated by incubating transfected HEK-293 cells with 50 pM [^{125}I]-hrIL-1β and varying concentrations of hrIL-1β. Free ligand was separated from bound by centrifugation as described [14]. Protein concentration was determined using the Lowry protein assay [15] with BSA as standard.

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PII S0014-5793(98)00467-0

2.4. Western blots

HEK-293 cell extracts (150 μg of protein), in non-reducing loading dye were separated on a 10% (w/v) SDS-PAGE, and blotted onto Nylon membranes (Amersham). The membranes were incubated with 0.35 μg/ml of the mAb 4E2 (rat anti-mIL-1RII), rabbit anti-rat-FITC (Daco A/S, Denmark, dilution 1:1000), and goat anti-rabbit-AP (Sigma, dilution 1:1000), respectively.

2.5. Facs analysis

HEK-293 cells and HEK-293 cells stably transfected with mIL-1RII-cDNA, were transiently transfected either with pcDNAI/mock vector or pcDNAI/mIL-1RAcP vector as described. Transfected cells were washed with PBS, 1 mM EDTA pH 7.45, incubated in non-enzymatic cell dissociation solution (Sigma) at 37°C, and washed twice in cold PBS pH 7.45, 0.05% (w/v) NaN_3 before incubation with or without mAb 4E2 (anti-mIL-1RII, 3.5 μg/ml). mAb 4E2 binding was detected by incubation with rabbit anti-rat-FITC (Daco A/S, Denmark, dilution 1:100 or 1:1000). The cells were washed twice in PBS pH 7.45, 0.05% (w/v) NaN_3, before FACS analysis.

2.6. Two hybrid IL-1R expression plasmids

The intracellular (ic) portion of the mIL-1RII, encoding aa 383–411 (icmIL-1RII), and the mIL-1RAcP, encoding aa 384–570 (icmIL-1RAcP), were obtained by RT-PCR. Total RNA were isolated from murine AtT-20 and murine GT1-7 cell lines, respectively. The resultant cDNA fragments were cloned into both the pGBT9 and the pGAD424 plasmids, sequenced and digested with restriction enzymes and shown to yield the expected restriction fragment sizes.

2.7. Yeast two hybrid screening

The yeast SFY526 (Clontech) reporter strain was utilised for the two hybrid studies according to the supplier's instructions. LacZ reporter gene transactivation was assayed of yeast colonies lifted from agar plates using Whatman No 1 filter paper quickly frozen in liquid nitrogen, and finally put on a filter paper submerged with phosphate buffered X-gal solution (pH 7.0, 0.33 μg/ml). The experiments were performed three times.

3. Results and discussion

The human kidney HEK-293 cells were transiently transfected with cDNA encoding the mIL-1RII, mIL-1RAcP, or cotransfected with both cDNAs. It is shown in Fig. 1A that cells transfected with the mIL-1RII-cDNA express a very low amount of [^{125}I]-hrIL-1β binding sites as compared to mIL-1RII/mIL-1RAcP-cDNA cotransfected cells. HEK-293 cells transfected with only mIL-1RAcP-cDNA did not bind [^{125}I]-hrIL-1β. The requirement of coexpression and complex formation between mIL-1RII and the mIL-1RAcP for the binding is further demonstrated by the inhibition of the [^{125}I]-hrIL-1β binding by antibodies directed against either the mIL-1RII (mAb 4E2) or by the antibodies directed against mIL-1RAcP (mAb 4C5), (Fig. 1A). The stoichiometric complex formation between the mIL-1RII and the mIL-1RAcP is further supported by the dose dependent appearance of specific [^{125}I]-hrIL-1β binding capacity when cells which are stably transfected and expressing the mIL-1RII, are transfected with increasing amounts of cDNA for mIL-1RAcP (Fig. 1B). We found that HEK-293 cells coexpressing mIL-1RII and mIL-1RAcP bind hrIL-1β with an IC_{50} of 2.5 ± 0.1 nM ($n = 3$) which is similar to the affinity for hrIL-1β of mIL-1RII on the murine pre-B-cell line 70Z/3 [3]. The 70Z/3 cell line endogenously expresses mRNAs for both the mIL-1RII and the mIL-1RAcP [16]. The very small amount of [^{125}I]-hrIL-1β binding sites upon mIL-1RII-cDNA transfection (Fig. 1A), which accounts to less than 10% of total binding obtained when compared to mIL-1RII/mIL-1RAcP-cDNA cotransfected cells was blocked by the mAb 4E2 (data not shown).

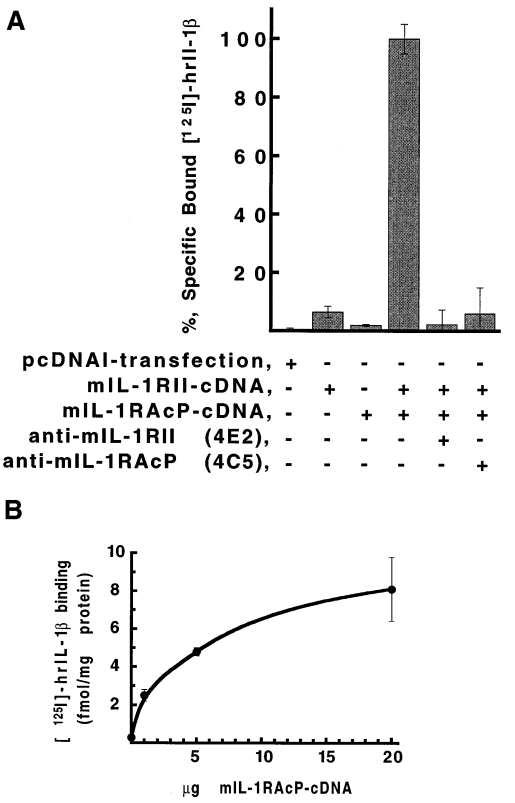

Fig. 1. Binding and inhibition of binding of [^{125}I]-hrIL-1β to the HEK-293 cell line expressing mIL-1RII and mIL-1RAcP. A: Specific binding data are shown for [^{125}I]-hrIL-1β binding to HEK-293 cells transiently transfected with the indicated cDNAs (+). Inhibition of binding of [^{125}I]-hrIL-1β by the mAb 4E2 to mIL-1RII (3.5 μg/ml) and the mAb 4C5 to mIL-1RAcP (10 μg/ml) is shown. 100% specific binding was defined as the specific binding to mIL-1RII/mIL-1RAcP-cDNA cotransfected HEK-293 cells and corresponds to 0.8 ± 0.3 fmol bound [^{125}I]-hrIL-1β/mg protein. Results are the mean ± S.D. of three independent experiments each based on duplicates. B: [^{125}I]-hrIL-1β binding by HEK-293 cells stably transfected with mIL-1RII-cDNA. Stably transfected cells were transiently transfected with 20 μg plasmid of graded concentrations of mIL-1RAcP-cDNA (0, 1, 5, and 20 μg). The figure is the mean ± S.D. of two independent experiments each based on duplicates.

To further investigate the role of mIL-1RAcP in the binding of hrIL-1β by the mIL-1RII, HEK-293 cells stably expressing the mIL-1RII (293-RII) were generated. The level of mIL-1RII expression in 293-RII cells was examined both by Western blot and FACS analysis using mAbs (4E2) to the mIL-1RII. It is shown in Fig. 2A that transient transfection with mIL-1RAcP-cDNA of 293-RII cells does not alter the level of mIL-1RII expression as compared to pcDNAI transfection. To examine whether the mIL-1RII is expressed on the cell surface in the absence of the IL-1RAcP we examined the stably transfected 293-RII cells under conditions of transfection with pcDNAI or mIL-1RAcP-cDNA. Fig. 2B present FACS data showing that the same amount of immunoreactive mIL-1RII is expressed on the cell surface, both in the absence and presence of the mIL-1RAcP. Even though expression of mIL-1RAcP did not alter cell surface expression of the mIL-1RII, a more than 20-fold increase in the number of [^{125}I]-

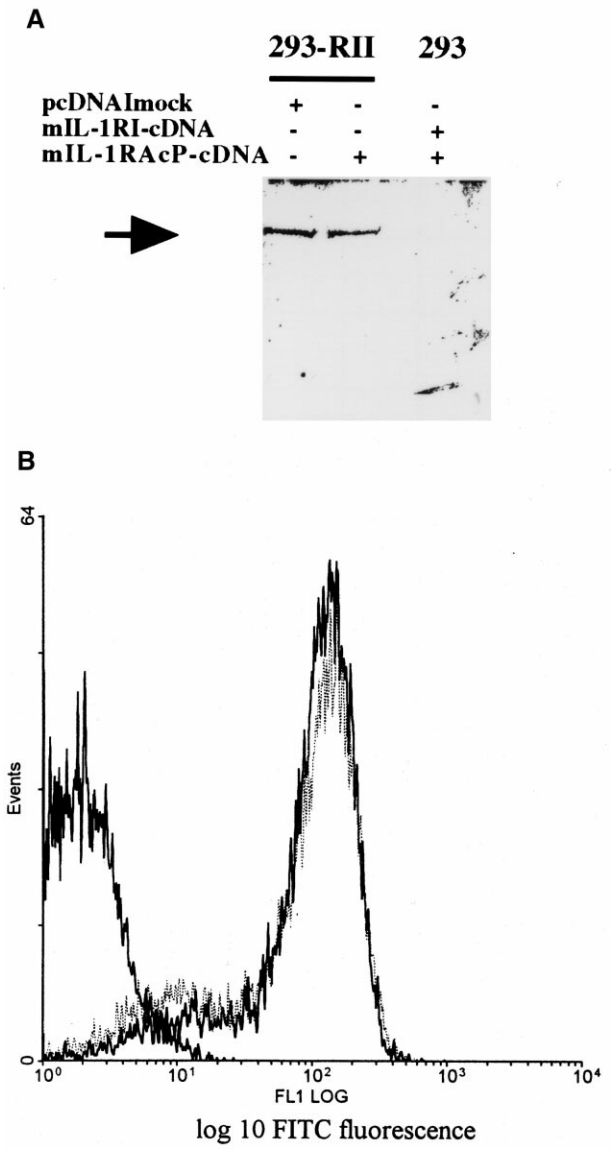

Fig. 2. Expression of the mIL-1RII by HEK-293 cells stably transfected with the mIL-1RII-cDNA. A: Western blot using the mAb 4E2 (anti-mIL-1RII). HEK-293 cells stably transfected with mIL-1RII-cDNA (293-RII) were transiently transfected with the indicated cDNAs (+). Control wild-type HEK-293 cells (293) transiently co-transfected with the indicated cDNAs (+) do not express mAb 4E2 immunoreactive protein (mIL-1RII). Arrow indicates 68 kDa. B: FACS analysis of the mIL-1RII cell surface expression using the mAb 4E2. The rat mAb 4E2 binding was visualised by incubation with rabbit anti rat-FITC (Daco A/S, Denmark). HEK-293 cells stably transfected with the mIL-1RII-cDNA were transiently transfected with mock vector pcDNAI (solid line) or mIL-1RAcP-cDNA (dashed line). mIL-1RAcP-cDNA transfected cells, incubated without mAb 4E2, is shown in shaded. Control HEK-293 cells transfected and incubated as above showed no immunoreactivity with mAb 4E2 (data not shown).
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hrIL-1β binding sites was observed in cells expressing both mIL-1RII and mIL-1RAcP (Fig. 1B). This means that the IL-1RII alone has an affinity lower than that detectable by us using 50 pM [^{125}I]-hrIL-1β as tracer.

Equilibrium binding experiments by McMahan and colleagues showed that the K_d of [^{125}I]-hrIL-1β for the recombinant mIL-1RII such as expressed on the green monkey fibroblast-like cell line CV1/EBNA was 20 nM [3]. Furthermore, using 300 pM of [^{125}I]-hrIL-1β as tracer in displacement binding experiments, 20% of the specific bound [^{125}I]-hrIL-1β could be displaced by hrIL-1β with an IC_{50} of 1.25 nM, while 80% of the bound [^{125}I]-hrIL-1β was displaced with an $IC_{50} > 100$ nM [3]. Gene transfer studies using human IL-1RII-cDNA [12,17] suggest that the low affinity of the hrIL-1β to the membrane bound mIL-1RII on HEK-293 cells is due to species specific differences in receptor affinity. However, since the expression levels of the IL-1RAcP usually are not investigated, it is still possible that membrane IL-1RII in general, have low affinity for the IL-1 family of ligands in the absence of the IL-1RAcP.

We also examined whether the IL-1RII-IL-1RAcP complex required for hrIL-1β binding is preformed – in the absence of an agonist – through direct interaction between the intracellular domains of the transmembrane receptors. Using the yeast two hybrid system we could not detect any interactions between the intracellular portions of IL-1RII and IL-1RAcP (Table 1). Neither did we detect any interaction between IL-1RII receptors or IL-1RAcPs that would suggest dimerisation of these in absence of their extracellular domains and/or in the absence of the agonist (Table 1). Control experiments showed clearly that the two hybrid system functions (Table 1).

We have performed similar cotransfection experiments on HEK-293 cells as those in Fig. 1A, using cDNA for the mIL-1RI and the mIL-1RAcP, respectively and found that the type I IL-1R (IL-1RI) can bind [^{125}I]-hrIL-1β with an IC_{50} of 1.6 ± 0.2 nM ($n = 3$) and when the cells also express the IL-1RAcP the binding affinity increases (IC_{50} 0.5 ± 0.2 nM) ($n = 3$). Thus we confirm earlier published data showing that the IL-1 binding to the mIL-1RI is possible in the absence of the mIL-1RAcP [18], and that presence of mIL-1RAcP will increase the affinity of the mIL-1RI for hrIL-1β approximately 5-fold [5]. This fact is of importance evaluating the significance of the present findings, since our data show that for binding of an agonist to the IL-1RII the presence of an IL-1RAcP is required, thus the IL-1RII will compete with the IL-1RI for IL-1RAcP in the presence of an agonist and there-

Table 1
Intracellular IL-1R interactions in the yeast two hybrid system

	icIL-1RII pGBT9	icIL-1RAcP pGBT9	pVA3
icIL-1RII pGAD424	–	–	-
icIL-1RAcP pGAD424	–	–	-
pTD1	-	-	+

The intracellular portion of the mIL-1RII (aa 383–411) and of mIL-1RAcP (aa 384–570) was expressed as fusion proteins with the GAL4 DNA binding (encoded in pGBT9) and transcriptional activation (encoded in pGAD424) domains, respectively. Interactions between intracellular portions of mIL-1RII and IL-1RAcP were measured as lacZ reporter gene activity. pVA3 and pTD1 are control plasmids supplied by the manufacturer, which yielded positive signal. The experiments were performed three times.

by may decrease the number of signalling IL-1RI-IL-1RAcP complexes per cell, and dampen the response to IL-1 agonists. This is an additional mode of modulating IL-1 signalling via the IL-1RII.

It is interesting to note that the cross-linking studies on soluble IL-1RII-IL-1 complexes suggest that the soluble type II IL-1R (sIL-1RII) does bind the agonist in the absence of the extracellular domain of the IL-1RAcP [17], thus it appears that while anchored to the membrane, the IL-1RII modulates signalling both via availability of the IL-1RAcP and via binding IL-1 agonists. The relative importance of the shedding of the IL-1RII and of the complexing and sequestering the IL-1RAcP is not possible to determine from these studies in absence of data on the endogenous abundance of IL-1RI, IL-1RII and the IL-1RAcP, respectively and without data on the relative affinities of the IL-1RI and IL-1RII for the IL-1RAcP. Our data show qualitatively that cell surface expressed IL-1RII may act to inhibit the IL-1 signalling via binding the IL-1RAcP.

Acknowledgements: The authors are grateful to Drs. R. Chizzonite, M. Labow and G. Ju at Hoffmann-La Roche for providing us with the mAbs 4E2 and 4C5, and to Dr. J.E. Sims at Immunex for providing us with the pRep3/mIL-1RII. Ms. Gun Jönsson at the Department of Immunology at Stockholm University is thanked for assistance with FACS analysis. This study was supported from grants from the Swedish Medical Research Council and EU Biomed Concerted Action (FMRX-CT97-0149). S.L. was supported by INSERM and IPSEN.

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