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Co-expression of mRNA for type I and type II Interleukin-1 receptors and the IL-1 receptor accessory protein correlates to IL-1 responsiveness

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Abstract Three cell surface molecules participate in Interleukin-1 (IL-1) binding and signal generation, the two distinct types of receptors (type I IL-1R and type II IL-1R) and the IL-1 receptor accessory protein (IL-1RAcP). Low surface expression hampers the detection of all three components on a protein level in most cell types, thus the highly sensitive RT-PCR was used to analyse the mRNA expression in a panel of 18 murine cell types of different hemopoietic lineages and fibroblasts. The transcription of both types of IL-1 receptors was detected in all cell lines tested. In most cell lines the IL-1RAcP was co-expressed with the IL-1 receptors, and only these lines responded to IL-1. However, in three cell lines no mRNA for the IL-1RAcP could be detected, and these cells did not respond to IL-1. These results suggest that the expression of the IL-1RAcP correlates with IL-1 responsiveness and they point to a pivotal role for the IL-1RAcP in IL-1 signal generation.

Key words: Interleukin-1 receptor (IL-1R); IL-1 receptor accessory protein (IL-1R AcP); Mouse hemopoietic cell line; Gene expression

1. Introduction

Interleukin-1 (IL-1) is an important pro-inflammatory cytokine with local and systemic effects resulting in a multitude of biological responses by a wide variety of target cells [1]. As a polypeptide mediator IL-1 exerts its function by binding to specific plasma membrane receptors.Two distinct forms of receptors for IL-1 have been cloned in mammalian cells, the type I IL-1R with a molecular mass of 80 kDa [2] and the type II IL-1R with a molecular mass of 68 kDa [3]. Whereas the type I IL-1R binds IL-1 and generates a signal in the target cell, the type II IL-1R binds the ligand but does not lead to an intracellular response. Its function has been discussed as a ligand sink to distract IL-1 from the type I IL-1R, thus serving rather as a decoy receptor [4,5]. The type II IL-1R can exert this function in a plasma membrane anchored form and in a soluble form [6].

A cell type specific expression of these two types of IL-1 receptors was reported. T-cells and fibroblasts express mainly type I IL-1R, whereas B-cells and monomyeloic cells bear predominantly type II IL-1R. Older reports state that some cell lines express one type of receptor exclusively, whereas recent data suggest that a co-expression of both types of IL-1 receptors is possible [7]. Very recently, an IL-1 receptor accessory protein (IL-RAcP) has been identified and cloned [8]. This molecule does not bind IL-1 itself but seems to enhance the affinity of the type I IL-1R for its ligand, suggesting that the IL-1R is a multi-subunit complex.

We addressed the question whether IL-1 receptors are co-expressed and whether the expression of the IL-1RAcP correlates with the responsivess of cells to IL-1. In all 18 murine cell lines tested a coexpression of mRNA of both types of receptors was detected by RT-PCR, and most, but not all cell lines expressed mRNA for the IL-1RAcP. Interestingly, the cell lines lacking mRNA for the IL-1RAcP were found to be unresponsive to IL-1, as ascertained by the two most common early IL-1 effects known from literature: NFkB activation and activation of the IL-1 receptor associated protein kinase IRAK [9,10], demonstrating a strong correlation of IL-1 responsiveness and expression of IL-1RAcP.

2. Materials and methods

2.1. Reverse transcriptase polymerase chain reaction (RT-PCR)

2.1.1. Reverse transcriptase reaction. RNA from 10^7 cells was extracted using RNA-Zol (AGS, Heidelberg). The isolated RNA was treated with DNase I (Boehringer Mannheim) using standard conditions. The RNA was extracted by a phenol/chloroform/isoamyl alcohol treatment [11]. Reverse transcription was carried out in a final volume of 10 µl per sample. For each reaction 1 µl reverse-transcription buffer (Stratagene), 1 µl DTT solution (100 mM, Stratagene), 5.5 µl DEPC water, 1 µl dNTPs (10 mM of dATP, dCTP, dGTP, dTTP each, New England Biolabs), 0.5 µl random hexamers (100 µM, Pharmacia), 0.5 µl reverse transcriptase (50 U/µl, Stratagene) and 0.5 µl template RNA (1 µg/µl) were mixed on ice, overlaid with mineral oil (Sigma) and incubated in a thermocycler (Vario V, Landgraf, Hannover) with a program of 60 min at 37°C, 5 min at 99°C and 5 min at 5°C. As negative control the reverse transcriptase was substituted with DEPC-water.

2.1.2. Polymerase chain reaction. The cDNA was amplified with three different pairs of primers. IL-1RI (product length: 363 bp), primer 1: CTG GAG ATT GAC GTA TGT ACA GAA TAT CCA AAT; primer 2: ATC CCC GGC AAT GTG GAG CCG CTG TGG GAA GGT GGC CTG TGT. IL-1RII (product length: 414 bp), primer 1: TTC ACC ACT CCA ACA GTG GTG CAC ACA GGA; primer 2: CAA GTA GGA GAC ATG AGG CAG AGA TGC TTC AGT. IL-1RAcP (product length: 677 bp), primer 1: AAC CAT CGG TCA CTT GGT ATA AGG G; primer 2: TTC ATC TGT TCC AAA GTG AGC TCG G. The reactions were performed in a final volume of 50 µl. 10 µl cDNA solution were added to a mixture of 1.6 µl magnesium chloride (50 mM, GibcoBRL), 4 µl PCR buffer (GibcoBRL), 0.8 µl dNTPs, 25.4 µl DEPC water, 0.2 µl Taq polymerase (GibcoBRL) and 4 µl of each primer 1 and primer 2 (10 µmol/l). The samples were overlaid with mineral oil (Sigma) and placed in a Vario V thermocyler (Landgraf). Program: 5 min at 95°C, 40 cycles with 1 min at 95°C, 1 min annealing (65°C for IL-1RI and IL-1RAcP, 55°C for IL-1RII), 1 min at 72°C followed by 7 min at 72°C. The PCR products were analysed on a 1.5% agarose gel and detected via ethidium bromide staining [11]. Specificity of the resulting PCR products was ascertained by performing a restriction digest with *Hin*fI (20000 U/ml, AGS, Heidelberg).

2.2. Measurement of IL-1 responses

IRAK activation was measured in type I IL-1R immunoprecipitates in an in vitro kinase assay as described recently [9]. D10N proliferation assays were performed as described [12]. For determination of PGE2 cells were grown to confluency and incubated with or without

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10 ng/ml IL-1β for 24 h. The supernatants were centrifuged (8 min, 1200 rpm) and analysed by ELISA as described earlier [13]. Interleukin-2 (IL-2) was measured in a bioassay using the IL-2 dependent murine cytotoxic T cell line CTLL [14].

2.3. Electrophoretic mobility shift assays (EMSA)

Nuclear extracts from 2×10^7 cells were obtained using a standard protocol (e.g. [15]), including a Nonidet P-40 solubilization step. Binding reactions were carried out with 5 μg protein. An oligonucleotide containing the NFκB binding sequence (TGA CAG AGG GGA CTT TCC AGA GA [15]) was radiolabeled with the T4 polynucleotide kinase (Pharmacia, Freiburg). For the binding reaction 5 μg nuclear extract (1 μl) was preincubated with (for supershift) or without 1 μl c-rel antibody (0.1 μg/μl, Santa Cruz) and 4.5 or 5.5 μl H_2O for 30 min at room temperature. This mixture was incubated with 1 μl of the radiolabelled double-stranded oligonucleotide (~0.1 pmol with 12000 cpm), 1 μl competitor DNA (1 μg/μl poly(dIdC)) and 1 μl HP buffer (10 mmol/l Tris, 10 mmol/l EDTA, 10 mmol/l DTT, 500 mmol/l NaCl, 50% glycerol, pH 7.5) for 30 min at room temperature. The binding reaction was stopped by adding 2 μl stop buffer (0.5% bromophenol blue, 50% glycerol). The protein/DNA complexes were separated in a polyacrylamide gel (4%).

3. Results

3.1. Co-expression of mRNA of type I and type II IL-1 receptors

A panel of 18 murine cell lines of different hemopoietic lineages and fibroblasts was analysed for gene expression of the two types of IL-1 receptors in a qualitative manner. The selected primers for the type I IL-1R led to the amplification of a 363 bp product (Fig. 1). The primers designed for the type II IL-1R resulted in a 414 bp product (Fig. 2). Both products were detected in all cell lines tested (summarized in Table 1). *Hin*fI digestion of these PCR products resulted in the fragments expected from the sequence data, confirming the specificity of the two products (data not shown). Contamination of the RNA preparations with genomic DNA was excluded by performing a DNAse digestion step and by including negative controls in the absence of reverse transcriptase. In these negative controls no signals were detected.

3.2. IL-1RAcP is not expressed in all IL-1 R positive cell lines

In most of the cell lines expressing IL-1 receptors a signal of 617 bp could be detected for the mRNA of the IL-1RAcP (Fig. 3). Restriction digests of this PCR product yielded the expected fragments, again demonstrating the specificity of the signal obtained in the RT-PCR (data not shown). However, in the monomyeloic cell line FDCP1, and in the thymomas S49 WT and S49 Cyc^- no signal was detected (Fig. 3 and summarized in Table 1). Inclusion of aldolase-specific primers as positive controls (aldolase) resulted in a strong signal of 464 bp in all reactions performed (data not shown), proving that false negative results due to degradation of RNA can be excluded.

3.3. Correlation of IL-1RAcP expression and IL-1 responsiveness

In order to check the biological implications of the lack of IL-1RAcP expression, the IL-1 reactivity of those cells which do not express the IL-1RAcP was tested. In contrast to control cells (EL-4 6.1, D10N, L929, Swiss 3T3), an IL-1 induced activation of IRAK could not be detected in the cell lines FDCP1 and S49 (data not shown). However, as IRAK activation is measured in immunoprecipitates of type I IL-1R this assay may be too insensitive in cells expressing very low numbers of receptors. Thus, an EMSA with an oligonucleotide probe specific for NFκB was performed as a more sensitive technique. In nuclear extracts of EL4 6.1 cells a very strong IL-1 induced activation of a NFκB-like binding activity was easily observed. A supershift experiment with an anti-c-rel antibody, a component of the NFkB complex, shows the con-

Table 1
Summary of the RT-PCR analysis of the IL-1 receptor components

Cell line	Cell type	Source of cells[a]	IL-1 RI		IL-1 RII		IL-1 RI AcP	
			Transcript[b]	Number[c]	Transcript[b]	Number[c]	Transcript[b]	Number[c]
70Z/3	pre B-cell	ATCC	+	3	+	3	+	2
SPGM1 2D8	pre B-cell	WEHI	+	4	+	3	+	2
SPGM1 x bcl	pre B-cell, bcl transfected	WEHI	+	2	+	2	+	2
Wehi 231	B-cell	WEHI	+	6	+	3	+	2
FDCP1	promonocyte	WEHI	+	3	+	2	−	4
2D8D	macrophage	ATCC	+	2	+	3	+	2
H 4-7	macrophage	ATCC	+	2	+	3	+	2
P388 D1	macrophage	ATCC	+	3	+	2	+	2
PU 5-1.8	macrophage	ATCC	+	2	+	1	+	2
RAW 264	macrophage	ATCC	+	3	+	2	+	2
Wehi 3B	macrophage	WEHI	+	2	+	3	+	2
BW 5147	T-cell lymphoma	ATCC	+	6	+	2	+	2
D10N	T-helper cell (TH2)	SH	+	20	+	51	+	5
EL-4 6.1	T-cell lymphoma	RMcD	+	11	+	3	+	12
S49 WT	T-cell lymphoma	ATCC	+	3	+	1	−	4
S 49 Cyc−	T-cell lymphoma, adenylate cyclase deficient	GS	+	2	+	2	−	2
L929	fibroblast	DSM	+	4	+	2	+	2
Swiss 3T3	fibroblast	DSM	+	3	+	2	+	2

[a]Source of cell line – ATTC: American Type Culture Collection, Rockville, Maryland, USA; WEHI: The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia; SH: kind gift of S. Hopkins, Manchester, UK; RMcD: kind gift of R. McDonald, Lausanne, Switzerland; GS: kind gift of G. Schultz, Berlin, Germany; DSM: Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany.
[b]Signal intensity: −, no signal; +, signal.
[c]Number of positive or negative proofs.

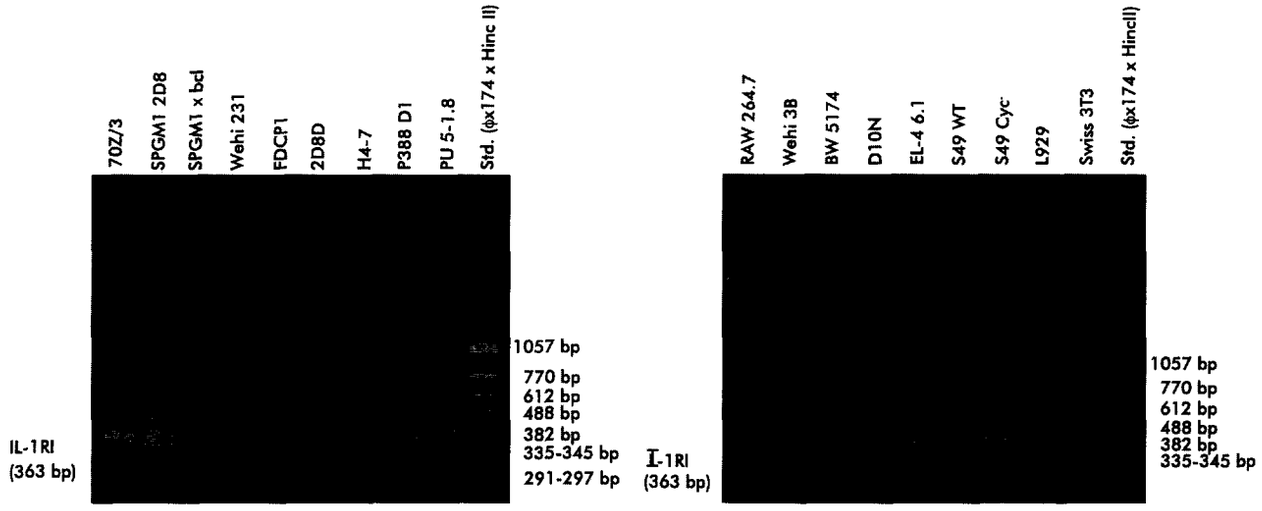

Fig. 1. Agarose gel electrophoresis with the type I IL-1 receptor RT-PCR products.

tribution of this molecule in the IL-1 induced DNA binding activity (Fig. 4). Neither FDCP1 nor S49 WT showed any IL-1 induced NFkB binding capacity. A weak basal binding activity in nuclear extracts of these cell lines was not influenced by IL-1 treatment and is probably due to signalling pathways activated by growth factors necessary for the proliferation of these cell lines (e.g. IL-3). In parallel, assays were performed with cell lines expressing IL-1RAcP mRNA, which proved to be IL-1 responders. We observed IL-1 induced IL-2 production in EL-4 6.1, IL-1 dependent proliferation of D10N, and IL-1 induced PGE2 production of Swiss 3T3 (summarized in Table 2).

4. Discussion

Presently, it is unclear which molecules of the IL-1 receptor complex actually define the IL-1 responsiveness of cells. On the plasma membrane three molecules are involved in IL-1 binding, the two types of receptors and the IL-1R accessory protein. The type I IL-1 R binds IL-1 and initiates signal transduction, whereas the type II IL-1R only binds and supposedly distracts IL-1 from the type I IL-1R [16,17], thereby elevating the threshold of IL-1 concentration necessary to elicit effects. This is suggested by experiments in cell lines possessing a given number of type I IL-1R in which overexpression of type II IL-1R results in a shift of IL-1 dose-response curves to higher concentrations [18]. However, not only may this distraction of ligand by type II IL-1R play a role in defining the sensitivity of cells to IL-1, but also the presence and level of expression of the IL-1RAcP, as suggested by the results described here.

Thus, the aim of this study was two-fold. We wanted to clarify whether co-expression of the two types of IL-1 receptors is common and whether a correlation can be found between IL-1 response of cells and expression of the IL-1RAcP.

Firstly, the screening of murine cell lines of different hemopoietic lineages and fibroblasts shows that the mRNAs for both types of IL-1 receptors are widely co-expressed. In fact, we could not find any cell lines which synthesized either one species of mRNA for IL-1 receptors or none at all. Qualitative RT-PCR does not allow for quantitation of the achieved results, so neither a statement on the ratio of mRNA species which is expressed in a given type of cell can be made, nor can anything be stated concerning levels of protein expression. However, as mRNA expression is a prerequisite for protein synthesis, the assumption can be made that functional protein is expressed. As most cell lines and especially normal tissue cells express extremely low numbers of IL-1 receptors on their surface, detection and distinction of the two types of receptors is practically often unfeasible. In some cells in which both mRNAs are present, both types of IL-1 receptors can be visualized by crosslinking iodinated IL-1α, such as in D10N cells, in others only type I IL-1R can be demonstrated (e.g. EL 4) [7], and in still others such as 70Z/3 only type II IL-1R can be shown by this technique, yet 70Z/3 can respond to IL-1 [15,19], thus indicating that

Table 2
Correlation of IL-1RAcP expression and IL-1 responsiveness

Cell line	IL-1RAcP expression	IL-1 mediated signalling effects		Other IL-1 mediated effects
		IRAK activation	NFκB shift	
D10N	+	+	+	proliferation
EL-4 6.1	+	+	+	IL-2 production
FDCP1	−	−	−	not tested
L929	+	+	not tested	not tested
S49 WT	−	−	−	not tested
Swiss 3T3	+	+	not tested	PGE_2 production

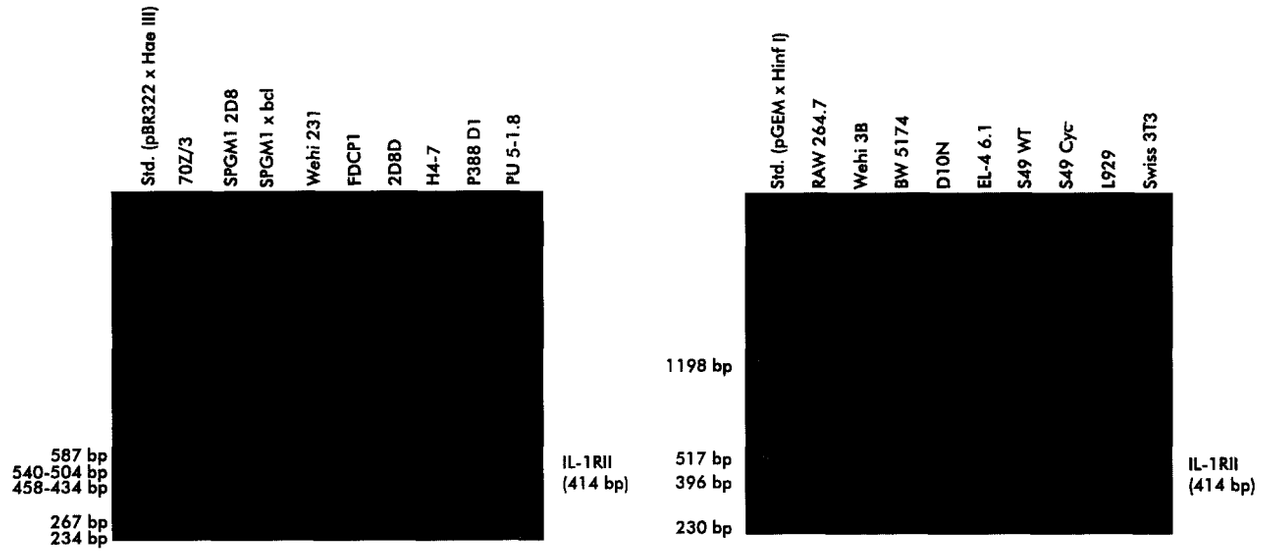

Fig. 2. Agarose gel electrophoresis with the type II IL-1 receptor RT-PCR products.

functional type I IL-1R must be present. Therefore, RT-PCR seemed to be the only technique to measure IL-1 receptor expression with a sensitivity high enough to achieve results at all.

Secondly, cells lacking IL-1RAcP seem to be unable to respond to IL-1.

IL-1 responsiveness can be measured at different levels. One of the first events measurable after IL-1 binding to the type I IL-1R is the activation of IRAK, the IL-1R associated serine/threonine protein kinase [9,10]. Further downstream in the IL-1 induced signalling cascade NFkB activation and translocation to the nucleus is a common event observed in many cell types (reviewed in [20]). In addition, biological effects such as IL-1 dependent proliferation of D10N cells, IL-1 induced IL-2 synthesis in EL-4 cells or prostaglandin production in fibroblasts can be measured. As no information was available on IL-1 induced effects for the cell lines S49 and FDCP1, which are negative for IL-1RAcP, we tried to measure IRAK activation by an in vitro kinase assay with type I IL-1R immunoprecipitates and NFkB activation by performing EMSAs, respectively. No effect of IL-1 could be observed in these cell lines, whereas IL-1 induced the expected effects in control cells, which expressed IL-1RAcP mRNA. This suggests that IL-1RAcP has to be expressed to facilitate signal transduction by the type I IL-1R. The fact that an antibody raised against the IL-1RAcP may abrogate an IL-1 response as described [21] strongly supports the notion that IL-1 RAcP is an essential co-receptor molecule in the signal generating complex of the type I IL-1 receptor.

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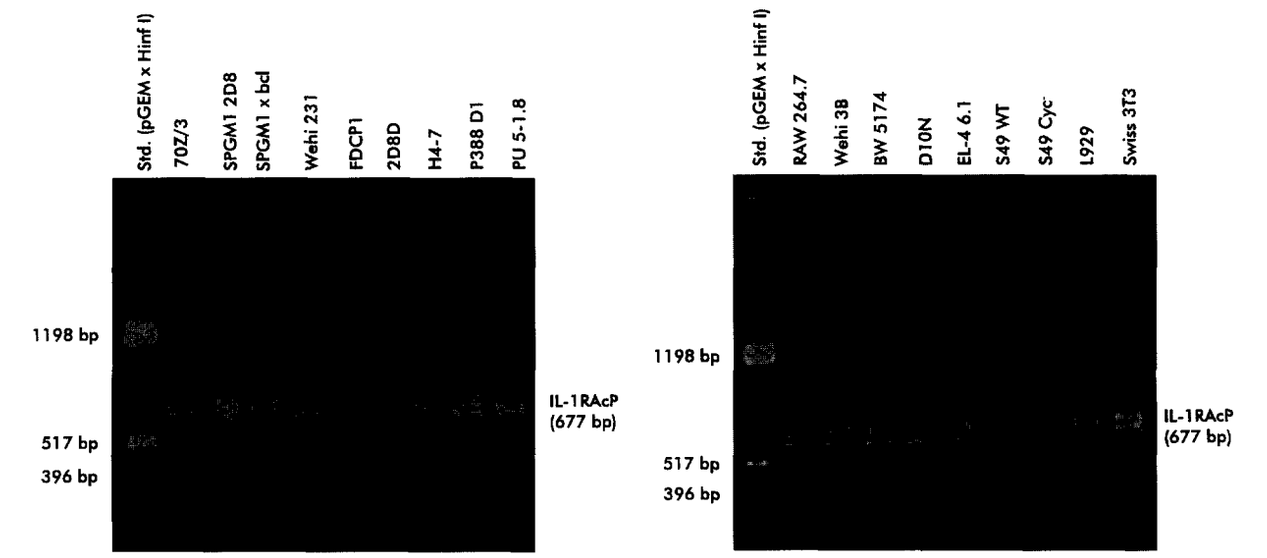

Fig. 3. Agarose gel electrophoresis with the IL-1RAcP RT-PCR products.

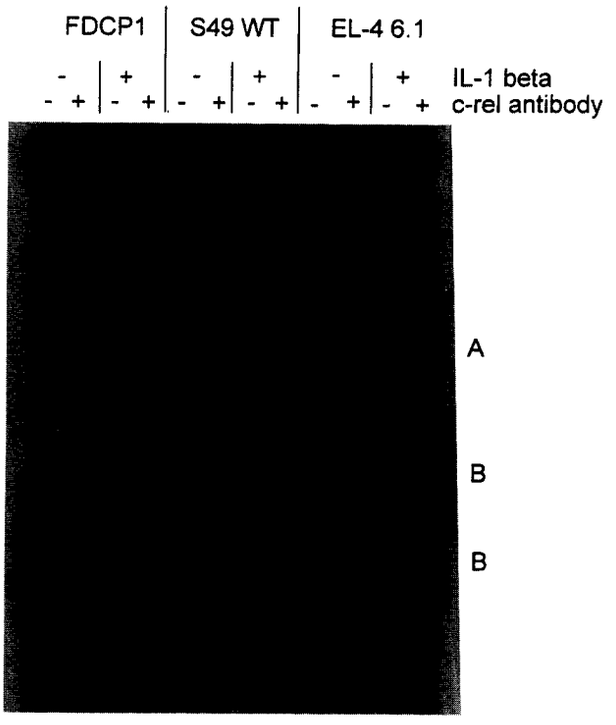

Fig. 4. IL-1 does not induce NFκB activation in IL-1RAcP deficient cell lines. The two IL-1RAcP deficient cell lines (FDCP1 and S49 WT) and the IL-1 responder EL-4 6.1 were incubated without IL-1 (−IL-1) or with 10 ng/ml of rhIL-1β (+IL-1). Nuclear proteins were prepared, incubated with a radiolabeled double-stranded cDNA containing the NFkB binding motif, and an EMSA was performed. IL-1 induced a strong shift in EL-4 6.1, resulting in two bands (bands B), which was not observed in FDCP1 or in S49 WT. Supershift experiments using an anti-crel antibody identified the complex and demonstrated the participation of crel as a component of the NFκB-like binding activity (band A).

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