

Model of interaction of the IL-1 receptor accessory protein IL-1RAcP with the IL-1 β /IL-1R_I complex

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Abstract A preliminary model has been calculated for the activating interaction of the interleukin 1 receptor (IL-1R) accessory protein IL-1RAcP with the ligand/receptor complex IL-1 β /IL-1R_I. First, IL-1RAcP was modeled on the crystal structure of IL-1R_I bound to IL-1 β . Then, the IL-1RAcP model was docked using specific programs to the crystal structure of the IL-1 β /IL-1R_I complex. Two types of models were predicted, with comparable probability. Experimental data obtained with the use of IL-1 β peptides and antibodies, and with mutated IL-1 β proteins, support the BACK model, in which IL-1RAcP establishes contacts with the back of IL-1R_I wrapped around IL-1 β . © 2001 Federation of European Biochemical Societies. Published by Elsevier Science B.V. All rights reserved.

Key words: Interleukin 1; Receptor chain; Accessory protein; Molecular modeling

1. Introduction

The cytokine interleukin 1 (IL-1) induces multiple physiological responses to inflammation, infection, and tissue damage [1]. Its potent defense activity requires a tight control, to avoid unwanted pathological derangements [1]. Initiation of cell activation occurs upon binding of agonist ligands (IL-1 α or IL-1 β) to a specific membrane receptor, the transmembrane glycoprotein of the immunoglobulin (Ig) superfamily IL-1R_I [2]. Conversely, binding of the antagonist ligand IL-1ra does not initiate signaling. The mode of agonist vs. antagonist interaction has been clarified after crystallization and structure

resolution of IL-1R_I complexed with IL-1 β [3] and IL-1ra [4]. From these studies, it appears that the three Ig-like domains of IL-1R_I wrap around agonist IL-1 β , thanks to the flexible areas between the Ig-like domains. In the antagonist interaction with IL-1ra, only the first two receptor domains establish contact with the ligand, while the third domain remains free. Binding of IL-1 to IL-1R_I is not sufficient for initiation of cell activation. A second chain, the accessory protein IL-1RAcP, is necessary for IL-1-dependent signaling [5–7]. This chain, structurally very similar to IL-1R_I, does not bind IL-1 but interacts with the complex IL-1/IL-1R_I, possibly by establishing contact with the third domain of the receptor [8,9]. The approach of the cytosolic segments of IL-1R_I and IL-1RAcP initiates signaling. On the other hand, IL-1RAcP cannot bind to the antagonist complex IL-1ra/IL-1R_I and activation does not occur [5]. The mode of interaction between IL-1RAcP and the activating complex IL-1/IL-1R_I is still elusive, as the structure of IL-1RAcP is unknown. This study was therefore undertaken in order to predict a model for the trimeric activating interaction IL-1 β /IL-1R_I/IL-1RAcP. Identification of areas involved in the activating interaction will open new possibilities for targeted strategies of inhibition to be used in IL-1-dependent chronic inflammatory or autoimmune pathologies.

2. Materials and methods

2.1. Computational methods

Modeling of the IL-1RAcP was performed using the IL-1R_I protein (1ITB, PDB protein code, 0.25 nm resolution) as template. Sequence alignment was performed with CLUSTALW [10] and LALIGN (www.ch.embnet.org/software/LALIGN_FORM.html). Secondary structure prediction was performed with a neural network predictor implemented in-house [11]. The 3D model was computed with the program MODELLER [12] and its quality checked with PROCHECK [13]. The Autodock suite of programs was used to determine the interaction between IL-1RAcP and the IL-1 β /IL-1R_I complex [14]. The final configuration of the complex was selected on the basis of minimal energy values. Models were visualized with RASMOL [15].

2.2. Antibody production

The synthetic peptide LKDDKPTLQ (sequence 73–81 of human IL-1 β) was conjugated to keyhole limpet hemocyanin, and inoculated repeatedly with adjuvants in New Zealand White rabbits (for production of polyclonal antibodies) and in BALB/c mice (for generation of monoclonal antibodies), as described [16,17]. Rabbit serum IgGs were purified by protein G. For monoclonal antibody generation, popliteal

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Abbreviations: IL-1, interleukin 1; IL-1R, IL-1 receptor; IL-1RAcP, IL-1R accessory protein; Ig, immunoglobulin; LAF, lymphocyte-activating factor

lymph node cells were fused with murine myeloma cells P₃X63-Ag8.653, antibody-producing hybridomas were cloned and subcloned and antibodies screened for recognition of IL-1 β in radioimmunoassay (RIA) [17]. Purified polyclonal IgG (Vhp19) and the selected monoclonal antibody (Vhp19G; IgG₃) recognized both the 73–81 peptide and human recombinant IL-1 β in RIA, and natural IL-1 β in immunofluorescence on lipopolysaccharide-stimulated monocytes, but did not bind IL-1 β in Western blot. Anti-IL-1 β antibodies BRhC3 and BRhD2 [18,19] were used as controls.

2.3. Binding assays

The effect of antibodies on IL-1 β binding to IL-1R₁ was assessed on the murine T cell lines EL4-6.1 or D10.G4.1 [20]. Briefly, cells were exposed to ¹²⁵I-labeled IL-1 β (0.4 nM), which had been pre-incubated in the absence or in the presence of antibodies overnight at 4°C [18]. After 90 min at room temperature, cell-bound radioactivity was counted in a γ -counter. Non-specific binding was determined in the presence of a 1000-fold excess of unlabeled IL-1 β .

2.4. Biological assays

The effect of antibodies on the biological activity of IL-1 β was assessed in three different biological assays, i.e. the IL-1 β -dependent proliferation of murine thymocytes (lymphocyte-activating factor (LAF) assay) [21,22] and of the murine Th2 cell line D10.G4.1 (D10 assay) [22], and the IL-1 β -stimulated production of prostaglandin E₂ (PGE₂) by human F7100 fibroblasts (PGE₂ assay) [21]. Briefly, cells were exposed to IL-1 β (0.01–1 ng/ml in the LAF assay; 2–20 pg/ml in the D10 assay; 0.02–0.5 ng/ml in the PGE₂ assay) in the absence or in the presence of antibodies. After incubation at 37°C for 2–3 days, supernatants were collected for PGE₂ determination (by RIA; DuPont NEN, Boston, MA, USA) or cells pulsed with [³H]thymidine for 18 h for determination of proliferation.

3. Results and discussion

A model of IL-1RAcP was calculated on the basis of alignment of primary sequence and secondary structure with IL-1R₁, and then modeled on the crystal structure of IL-1R₁ bound to IL-1 β [3]. Two other IL-1R₁ structures were available, i.e. that of the complex with the antagonist IL-1ra [4] and that of the complex with the 21 amino acid long antagonist peptide AF10847 [23]. Both interactions are abortive in terms of cell activation, i.e. do not allow interaction with the IL-1RAcP and initiation of signaling. Their IL-1R₁ structures were thus not considered high probability templates for the IL-1RAcP model. The IL-1RAcP model structure is shown in Fig. 1A.

Docking of IL-1RAcP to the crystal structure of IL-1R₁ bound to IL-1 β was attempted with Autodock programs. As a control, the same attempt was made on the crystal structure of IL-1R₁ bound to IL-1ra and, as expected, no interaction could be calculated (data not shown). Two possible types of IL-1 β /IL-1R₁/IL-1RAcP interaction were calculated. The first type of interaction (FRONT model) places the IL-1RAcP chain across the IL-1 β /IL-1R₁ complex, partially covering the area of IL-1 β not involved in receptor binding (Fig. 1B). Conversely, the second type of interaction (BACK model) places IL-1RAcP across the back of the complex, engaging part of IL-1R₁ (in particular the second and third Ig-like domains) and parts of IL-1 β along the border of interaction with IL-1R₁ (Fig. 1C).

To discriminate between the two models, an experimental approach was designed. This implies, based on the model structures, the identification of areas of IL-1 β in close proximity to IL-1RAcP in one model and completely detached from IL-1RAcP in the other. One such area was identified as the stretch in position 73–81 in the mature IL-1 β sequence (LKDDKPTLQ). This area is in close vicinity to residues of IL-1RAcP in the FRONT model (average of 3.5 Å; see cyan area in Fig. 1B), whereas it is located at over 20 Å from the closest IL-1RAcP residues in the BACK model (see cyan area in Fig. 1C). A synthetic peptide corresponding to the sequence 73–81 was synthesized and used to immunize mice and rabbits, to generate antibodies of pre-determined specificity. These antibodies, dubbed Vhp19, recognize specifically and with a significant affinity both the immunizing peptide and the entire IL-1 β (Table 1). The ability of Vhp19 to interfere with IL-1 β binding to IL-1R₁ was assessed and compared to inhibition of IL-1 β biological activity. As shown in Table 1, Vhp19 did not affect the capacity of IL-1 β to interact with IL-1R₁. This is in agreement with the information gathered from the crystal structure of the IL-1 β /IL-1R₁ interaction, where it is clear that IL-1 β area 73–81 is not involved in receptor binding (see Fig. 1B,C). If the area recognized by Vhp19 is involved in the interaction with IL-1RAcP (as in the FRONT model), Vhp19 would be expected to inhibit IL-1 β biological effects. However, as shown in Table 1, Vhp19 is completely unable to inhibit either IL-1 β -induced lymphocyte proliferation (LAF and D10 assays) or IL-1 β -induced production of

Table 1
Effect of antibodies of pre-determined specificity on IL-1 β functions

Antibody ^a	IL-1 β epitope recognized	Recognition of IL-1 β				
		Affinity in RIA (K_a , l/mol) ^b	Percent inhibition of binding to IL-1R ₁	Percent inhibition of activity in		
				LAF assay ^c	D10 assay ^c	PGE ₂ assay ^c
Unrelated	none	n.d.	6.5 \pm 2.0 ⁺	–4.9 \pm 3.8 ⁺	–13.2 \pm 2.9 ⁺	–21.3 \pm 3.5 ⁺
Vhp19	73–81	4.6 \times 10 ⁸	–5.6 \pm 4.2 ⁺	–5.3 \pm 3.3 ⁺	–2.4 \pm 4.3 ⁺	–10.6 \pm 3.9 ⁺
BRhC3	17–31	5.8 \times 10 ⁸	81.1 \pm 4.3*	78.0 \pm 3.1*	74.8 \pm 6.7*	69.9 \pm 3.3*
BRhD2	61–70	1.0 \times 10 ⁹	3.4 \pm 0.6 ⁺	68.6 \pm 3.8*	81.5 \pm 4.3*	n.t.

n.d., not detectable; n.t., not tested; ⁺not significant vs. control without antibodies; * P < 0.01 vs. control without antibodies.

^aUnrelated antibodies included monoclonal antibodies and polyclonal rabbit Ig to α -vimentin and to human growth hormone and normal rabbit Ig.

^bOn immobilized IL-1 β . Controls with unrelated proteins (IL-1 α , bovine serum albumin) or peptides (other IL-1 β peptides) yielded no reactivity.

^cMean \pm S.E.M. of data obtained with antibody concentrations ranging from 1 to 100 μ g/ml (IL-1R₁, LAF and D10) or from 50 to 250 μ g/ml (PGE₂) in 3–37 separate determinations. No inhibition of IL-1 α was detected. Mean control 100% values (in the absence of antibodies) were: for receptor binding 11 776 \pm 38 (cpm of specific IL-1 β binding), for LAF assay 30 069 \pm 5406 (cpm of specific IL-1 β -induced proliferation), for D10 assay 40 409 \pm 6687 (cpm of specific IL-1 β -induced proliferation), for PGE₂ assay 139.8 \pm 35.6 (specific IL-1 β -induced ng PGE₂/mg cell protein).

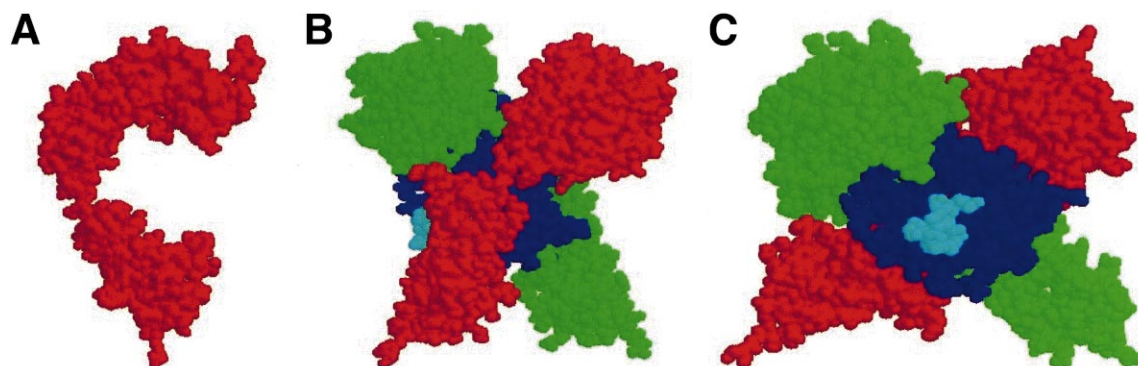


Fig. 1. Model of IL-1RAcP and of the complex IL-1 β /IL-1R₁/IL-1RAcP. The molecular model of IL-1RAcP (red) is depicted in A. The first and second Ig-like domains on the top appear as a compact structure, whereas the third domain, at the bottom, is clearly distinct. B represents the hypothetical FRONT model of interaction of IL-1RAcP (red) with the crystal structure of the complex of IL-1 β (blue) and IL-1R₁ (green). C represents the alternative BACK model of interaction. In B and C the cyan area represents the position of IL-1 β residues 73–81.

PGE₂ in fibroblasts (PGE₂ assay). To support these data, a series of mutations in area 74–80 did not affect IL-1 β binding to IL-1R₁ or biological activity [24]. In addition, the synthetic peptide corresponding to area 73–81 could not inhibit IL-1R₁ binding or biological activity of IL-1 β (unpublished). As a control, an antibody to an area involved in IL-1R₁ binding (BRhC3, recognizing an epitope within region 27–31) effectively inhibited both binding and biological activities of IL-1 β , whereas unrelated antibodies (to α -vimentin or to human growth hormone) were completely inactive. Eventually, another antibody (BRhD2, recognizing an epitope within the IL-1 β sequence 61–70) could not inhibit receptor binding but significantly decreased the biological effects of IL-1 β , thus possibly interfering with recruitment of IL-1RAcP to the IL-1 β /IL-1R₁ complex. Thus, although it cannot discriminate between the two models, IL-1 β area 61–70 looks very important in the interaction with IL-1RAcP [19]. Indeed, the double mutation of lysine residues in positions 63/65 to serine causes a significant impairment of biological activity without affecting binding to IL-1R₁ [27,28] (Table 2).

To gather further information which could allow discrimination between the two models, a literature survey was performed to identify IL-1 β mutants with discrete effects on receptor binding and biological activity [25]. Table 2 summarizes the few described mutations of amino acids on the external surface of the IL-1 β molecule (internal mutations have been excluded) which do not significantly affect binding to IL-1R₁ but profoundly impair biological activity (i.e. possibly involved in the interaction with IL-1RAcP). Calculations

of distance between these IL-1 β mutations and IL-1RAcP show that in the majority of instances these positions are much closer to IL-1RAcP in the BACK model than in the FRONT model. As already mentioned, one exception is the mutation K63S, which is in close proximity to IL-1RAcP in both models and therefore does not allow discrimination.

A series of other data are available, mainly obtained with antibodies raised against synthetic peptides, which help in the identification of interaction areas of IL-1 β [24], IL-1R₁ [32] and IL-1RAcP [8]. None of these data provide a clear indication to support one of the two proposed models. However, the experimental evidence is that IL-1RAcP interacts with IL-1R (bound to agonist IL-1 β) through its second and/or third domain [8,9]. Since IL-1RAcP is unable to directly bind IL-1 β with sufficient affinity [5], it could be hypothesized that complex formation between IL-1 β and IL-1R₁ leads to the creation of novel surface areas which include residues from both IL-1 β and IL-1R₁ and which can be bound with high affinity by IL-1RAcP.

This study therefore proposes a preliminary model to describe the activating interaction of IL-1RAcP with the complex IL-1 β /IL-1R₁. The model hypothesizes that IL-1RAcP establishes contacts with the back of IL-1R₁ wrapped around IL-1 β and with some parts of IL-1 β in the proximity of contact areas with IL-1R₁. A first validation of the model comes from experimental data obtained with synthetic peptides and specific antibodies. After further refinement and deeper experimental validation, the model may become the structural basis

Table 2
Mutations of IL-1 β affecting biological activity but not receptor binding

IL-1 β mutation	Effect on IL-1R ₁ binding ^a	Effect on biological activity ^b	Distance to IL-1RAcP ^c		Ref.
			FRONT model	BACK model	
D54R	none (EL4)	32 \times (LAF)	19 Å (from D257)	7 Å (from T274)	[26]
K63S	none/2–5 \times (EL4, chondr) ^d	6000–50 000 \times (LAF, chondr) ^d	3 Å (from F27)	2 Å (from F27)	[27,28]
K65S	none/2–5 \times (EL4, chondr) ^d	6000–50 000 \times (LAF, chondr) ^d	8 Å (from R10)	4 Å (from E28)	[27,28]
Y121K/A/G/L/T	none (EL4)	100–1000 \times , abolished (LAF, PGE ₂)	15 Å (from D272)	7 Å (from D267)	[29]
D145K	none (D10)	100 \times (LAF), abolished (PGE ₂)	21 Å (from L6)	3 Å (from K265)	[30,31]

^aMutants of IL-1 β were assayed for ability to displace binding of wild type IL-1 from the following IL-1R₁-bearing cells: EL4-6.1 (EL4), rabbit chondrocytes (chondr), D10.G4.1 (D10).

^bBiological assays included murine thymocyte proliferation (LAF), activation of rabbit chondrocytes (chondr), production of PGE₂ by human fibroblasts or smooth muscle cells (PGE₂).

^cCalculated minimal distance between the mutated amino acid and the closest residue of IL-1RAcP (in parentheses) in the two models.

^dData refer to the double mutation 63/65 K/K S/S. No data are available for the single mutations.

for designing targeted inhibitory strategies aimed at blocking IL-1 pathological activity in severe inflammatory diseases.

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