# NOVEL PATHWAYS FOR NEGATIVE REGULATION OF INFLAMMATORY CYTOKINES

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**ABSTRACT**

Inflammatory cytokines act in cascades. Pro and anti-inflammatory signals regulate the production of primary and secondary inflammatory cytokines. A number of studies have investigated the actual role played by the two receptors, RI and RII, in interleukin (IL)-1 signaling. All available evidences, including tissue distribution and monoclonal antibodies blocking studies, indicate that IL-1-induced activities are mediated exclusively via the IL-1RI, whereas IL-1RII has no signaling activity and inhibits IL-1 effects by acting as a decoy for IL-1, thus sequestering it and preventing the cytokine from binding to the IL-1RI. IL-1RII may represent a physiological pathway of inhibition of IL-1. Induction of expression and release of the IL-1RII may contribute to the antiinflammatory properties of Th2-derived cytokines and glucocorticoids. Other studies on chemokine receptors show that lipopolysaccharides cause a drastic and rapid downregulation of the expression of CCR2, a receptor for macrophage chemotactic proteins (MCP)-1 and -3.

*Keywords:* inflammation, interleukin-1, receptors, chemokines

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**RESUMEN**

Las citoquinas inflamatorias actúan en cascadas. Las señales pro y anti-inflamatorias regulan la producción de citoquinas inflamatorias primarias y secundarias. Varios estudios han investigado el verdadero papel que juegan los dos receptores, RI y RII, en la señalización de la acción de la interleuquina (IL)-1. Todas las evidencias disponibles, incluyendo estudios de distribución tisular y de bloqueo con anticuerpos monoclonales, indican que las actividades inducidas por IL-1 son mediadas exclusivamente a través del IL-1RI, mientras que el IL-1RII no tiene actividad de transducción de señal e inhibe los efectos de la IL-1 actuando como un desvío para la IL-1, secuestrándola y evitando que la citoquina se una al IL-1RI. El IL-1RII puede representar una vía fisiológica de inhibición de la IL-1. La inducción de la expresión y liberación del IL-1RII puede contribuir a las propiedades anti-inflamatorias de las citoquinas derivadas de las células Th2 y los glucocorticoides. Otros estudios sobre receptores de quimioquinas muestran que los lipopolisacáridos causan una regulación negativa drástica y rápida de la expresión del receptor CCR2, para las proteínas quimiotácticas de macrófagos (MCP)-1 y -3.

*Palabras claves:* inflamación, interleuquina-1, receptores, quimoquinas

## Introduction

Inflammatory cytokines act in cascades. One can schematically recognize primary inflammatory cytokines, the prototype of which is IL-1, and secondary effector molecules, among which chemokines play an important role in recruitment. Pro and anti-inflammatory signals regulate the production of primary and secondary inflammatory cytokines, sometimes in unexpected ways (1). The possibility that microenvironmental signals may regulate the action of proinflammtory cytokines by acting at the receptor level has been less extensively studied. Here we will review recent results on the action of pro and anti-inflammatory signals on receptors for IL-1, which in fact is a complex system and for the chemokine monocyte chemotactic protein-1 (MCP-1), a prototypic inflammatory cytokine.

## IL-1 receptors (IL-1R)

The first IL-1R was cloned from murine (2) and then human T cells (3). Soon after identification and cloning of this T cell IL-1R, thereafter named type IR (IL-1RI), it was evident that a second receptor does exist for IL-1, expressed in B lymphocytes and myelomonocytic cells, referred to as type II (IL-1RII) (4).

Transcripts of human and mouse IL-1RI are approximately 5 kb in length. A single open reading frame encodes for a protein of 552 amino acids (aa), with a molecular weight (MW) of, when fully glycosilated, 80-85 kD. The MW of the unglycosilated protein is 62 kD. On the basis of their structures, IL-1RI and IL-1RII have been assigned to the IgG-like superfamily of receptors, with the extracellular portion containing 3 IgG-like domains. The extracellular region (319 aa long), which contains 7 potential sites of N-glycosilation, of IL1RI is followed by a 20 aa long transmembrane region, and then by a 213 aa cytoplasmic portion. The cytosolic region has no homology with any kinase described

so far, and the only protein with which shows some homology is the Drosophila TOLL protein.

IL-1RII transcripts are approximately 1803 bp long. The human transcript encodes for a 386 aa protein of 68 kD. Treatment with N-glycosidases reduced the MW to 55 kD. Five potential sites of N-glycosylation have been identified. The extracellular region of 332 aa shares only 28 % homology with the corresponding region of IL-1RI in humans. A 26 aa transmembrane domain is then followed by a very short cytoplasmic domain of 29 residues (4).

As already mentioned, the genes of IL-1Rs are located on chromosome 2 (band q12-22) in humans and in the centromeric region of chromosome 1 in mice (4). The promoter region of IL-1RI has been recently described (5).

IL-1RI and IL-1RII are usually co-expressed. However, IL-1RI is expressed as the predominant form in fibroblasts and T cells. By contrast B cells, monocytes and polymorphonuclears (PMN) express preferentially the IL-1RII (4).

IL-1RI and IL-1RII have different affinities for the three ligands of the IL-1 family. Although some differences are evident among different studies, IL-1RI binds IL-1α with higher affinity than IL-1β (kd = $10^{-10}$ M and $10^{-9}$ M, respectively). By contrast, IL-1RII binds IL-1β more avidly than IL-1α (kd = $10^{-9}$-$10^{-10}$ M and $10^{-8}$ M, respectively). IL-1 receptor antagonist (IL-1ra) (6, 7) binds to IL-1RI with an affinity similar to that of IL-1α, whereas IL-1RII binds IL-1ra 100-fold less efficiently than IL-1RI (4).

Given the existence of two distinct IL-1R, a number of studies have investigated the actual role played by each of them in IL-1 signaling. As summarized briefly hereafter, all available evidences indicate that IL-1-induced activities are mediated exclusively via the IL-1RI, whereas IL-1RII has no signaling activity and inhibits IL-1 activites by acting as a decoy for IL-1 (8).

In a number of different cell types, circumstantial evidence has been obtained that different IL-1 activities are mediated by the IL-1RI. Human endothelial cells, in which IL-1 regulates functions related to inflammation and thrombosis, express exclusively IL-1RI, indicating at minimum that IL-1RII is dispensable for IL-1 signaling (9, 10). Blocking monoclonal antibodies (MAbs) directed against IL-1RI inhibited IL-1 activities in the hepatoma cell line HEPG2 (11), which expresses nearly equal amounts of IL-1RI and IL-1RII. IL-1α-induced co-stimulatory activity in CD4+ murine T cell clones, which express both receptors, was mediated solely by IL-1RI (12, 13). Similar results were obtained in keratinocytes (14). Using selective ligands, also fever was shown to be only mediated by IL-1RI (15).

IL-1RI appears to be the only signaling receptor also in cell types expressing predominantly the IL-1RII and only minute amounts of IL-1RI. Blocking MAbs against IL-1RI totally blocked IL-1- induced expression of cytokines and adhesion molecules in the human monocytic cell line THP-1, human circulating monocytes and PMN (11, 16). Also IL-1-induced survival of PMN was blocked by anti-type I blocking antibodies (16). A new IL-1R-related protein (accessory protein, or IL-1 AcP, or IL-1RIII) was recently cloned (17). It augments the affinity of IL-1RI for IL-1β (but not IL-1ra) and there is evidence that it plays a role in signal transduction (18, and Werner Falk, personal communication).

Whereas the signaling activity of IL-1RI and IL-1RIII/AcP is well established, unequivocal evidence supporting a signaling function of IL-1RII is still lacking. Blocking MAbs against IL-1RII did not inhibit the biological activities of IL-1 in a number of different cell types, including lymphocytes, monocytes, PMN and hepatoma cells (11, 16). In monocytes, anti-type I antibodies blocked IL-1 activities, whereas anti-type II antibodies did not block and rather augmented the responsiveness of cell to IL-1, consistently with a model in which the IL-1RII is an inhibitor of IL-1 (see below) (11, 16). In addition to lacking any signaling function, the IL-1RII is shed in a soluble (sIL-1RII) form. sIL-1RII was found in the supernatants of the B lymphoblastoid cell line Raji and of mitogen-activated mononuclear cells (8). IL-1RII is also released by cytokine- and dexamethasone-treated PMN and monocytes (16, 19-21). sIL-1RII is rapidly shed within minutes after treatment of PMN and monocytes with chemotactic stimuli and oxygen radicals, indicating that release of this receptor represents an aspect of the complex reprogramming of myelomonocytic cells in response to these mediators (22, 23). An alternatively spliced mRNA encoding a soluble IL-1RII was recently cloned (24).

The finding that the IL-1RII has no signaling function and it is shed in a soluble form, suggested that this molecule could act as an inhibitor of IL-1. We examined this hypothesis in human PMN, in which we found that IL-1 is a potent inducer of PMN survival in culture (25). Since IL-4 inhibited IL-1-mediated survival, and IL-4 upregulated IL-1RII expression and release in these cells (16), we reasoned that the inhibitory activity of IL-4 on IL-1 activity could be mediated by an upregulation of IL-1RII. The inhibitory activity of IL-4 was totally abrogated by the presence of blocking antibodies directed against IL-1RII, thus demonstrating that IL-1RII inhibits IL-1 activity (16). We proposed that the mechanism of inhibition is to act as a decoy target for IL-1, consisting in the fact that IL-1RII binds IL-1 without any signaling function, thus sequestering it and preventing the cytokine from binding to the IL-1RI, the only IL-1R with a cell signaling function.

Consistently with the decoy model of action of the IL-1RII, blocking antibodies to IL-1RII augmented

the activity of suboptimal concentrations of IL-1 on IL-1-induced expression of cytokines and adhesion molecules in human circulating monocytes (11, 16). The released decoy RII inhibits processing of the precursor for IL-1β (26).

To validate the decoy model of action of the IL-1RII, this receptor was overexpressed in type I-expressing human fibroblasts or keratinocytes. As expected, IL-1 activity was reduced in fibroblasts expressing high levels of IL-1RII. The inhibitory effect of transfected type II receptors was evident at suboptimal concentrations of IL-1, whereas saturating amounts of IL-1,overcame the IL-1RII-mediated inhibition of IL-1 activity (27, 28). The finding that, glucocorticoids (GC) and Th2-derived cytokines (IL-4 and IL-13) upregulate IL-1RII expression and release (16, 19, 20, 29) is in keeping with the concept that the IL-1RII may represent a physiological pathway of inhibition of IL-1. Induction of expression and release of the IL-1RII may contribute to the antiinflammatory properties of Th2-derived cytokines and GC.

### Regulation of chemokine receptor expression

MCP-1 is a prototypic C-C chemokine active on mononuclear phagocytes, basophils, T cells and NK cells (30-32). Other C-C chemokines, including the recently identified macrophage derived chemokine (MDC), are also active on dendritic cells (31, 33). We examined the effect of lipopolysaccharides (LPS) on chemokine receptor expression and we found that LPS causes a drastic and rapid downregulation of the expression of CCR2, a receptor for MCP-1 and -3. The ED50 of LPS was ≅ 1 ng/mL and half maximal effect was reached with an optimal dose in ≅ 45 min. Inhibition of MCP-1 receptor expression was functionally relevant since LPS-treated monocytes showed a reduced capacity to bind and to respond to MCP-1 chemotactically. The action of LPS on C-C chemokine receptors was specific in that CXCR2 was unaffected (34). In neutrophils, LPS and TNF-α were reported to inhibit the expression of IL-8 receptors, while G-CSF increased it (35). IL-2 was shown to induce CCR2 in T lymphocytes and NK cells (36-38), an observation confirmed here for monocytes. Interestingly, CCR2 induction in T cells was a slow process, requiring four days of exposure to the cytokine (36). The results reported here show a dramatic, rapid and differential downregulation of chemokine receptors by LPS in monocytes (34).

LPS did not inhibit the rate of nuclear transcription of CCR2, but did reduce the mRNA half life from 1.5 h to 45 min. Regulation of CCR chemokine receptor expression, in addition to agonist production, is likely a crucial point for regulation of the chemokine system. We speculate that the divergent effect of certain proinflammatory signals on agonist versus receptor expression may serve to retain mononuclear phagocytes at sites of inflammation, to prevent their reverse transmigration (39), and, possibly, to limit excessive recruitment.

## Concluding remarks

Primary and secondary inflammatory cytokines are highly regulated by diverse signals. Emphasis has largely been on how pro- and anti-inflammatory molecules affect cytokine production. The results summarized here obtained with IL-1 receptors and with the MCP-1 receptor indicate that receptor expression may represent a crucial regulatory element for the tuning of the action of primary and secondary inflammatory cytokines.

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