REVIEW

Regulation of interleukin-1 activity is enhanced by cooperation between the interleukin-1 receptor type II and interleukin-1 receptor accessory protein

John E. Sims and Dirk E. Smith

Amgen, 51 University Street, Seattle, WA 98101

Correspondence: John Sims, Amgen, 51 University Street, Seattle, WA 98101. Tel: (206) 265-4005 Direct, Fax: (206) 233-9733
Email: sims@amgen.com
Accepted on 03 April 2001

The cytokine interleukin-1 (IL-1) possesses profound inflammatory activities (see 1 for review). Local production of IL-1, in response to injury or infection, initially induces chemokine secretion, adhesion molecule expression and vasodilation, resulting in trafficking of leukocytes to the affected area as well as their activation. Production of nitrogen and oxygen radicals aids in combating infection, as does induction of the acute phase response, fever, and the syndrome known as "sickness behavior" (sleep, loss of appetite, social withdrawal) [2]. Local tissue responses include release of metalloproteinases, inhibition of cartilage synthesis, and bone resorption, all of which assist in clearing damage from infection or injury. In addition to these degradative activities, remodeling also ensues, leading to formation of fibrotic tissue (see e.g. 3).

Signaling through the IL-1 type I receptor is required for all actions of interleukin-1 [4]. The receptor is a single-spanning, type I transmembrane protein with three immunoglobulin domains extracellularly and a cytoplasmic portion exhibiting a fold shared with other members of the IL-1 and Toll-like receptor families, generally known as a TIR domain (for Toll/IL-1 Receptor) [5, 6, 7]. Binding of IL-1 to the receptor extracellular portion allows recruitment of a second, homologous subunit termed IL-1 receptor accessory protein, or AcP [8]. The heterodimeric association of TIR domains inside the cell allows recruitment of the adapter protein MyD88. MyD88 is comprised of two domains, a TIR domain, which presumably assembles with those of the IL-1R type I and AcP, and a death domain, which recruits the death domain-containing IL-1 receptor activated kinases (IRAKs)-1 and -4. IRAK-1 then associates with another adapter called TRAF6, which in turn leads to activation of several protein kinases (JNK, p38, ERKs, the IKK complex) and transcription factors (NFκB, AP1, ATF2, C/EBPβ) [9], which eventually results in changes in gene expression and cell physiology.

The biological activities of IL-1 (in fact, two closely related molecules, IL-1α and IL-1β, regulated differently but acting identically through the IL1R-I and AcP complex) described in the opening paragraph, are of great benefit to the host in combating infection or responding to injury. Nevertheless, when carried to excess, by either too strong or too long a response, they have the potential to cause more harm than good. Degradation of existing cartilage and bone, and prevention of resynthesis, is reminiscent of the pathology seen in rheumatoid arthritis, and indeed, an antagonist of IL-1 (IL-1Ra or anakinra, see below) is of proven benefit in RA [10]. Excessive scar formation and/or fibrosis is a problem in many diseases, including asthma and congestive heart failure. The vasodilating and pyrogenic effects of IL-1 can be lethal, in an acute setting. Of necessity then, multiple means of regulating the action of IL-1 have evolved. Included in these are regulation of the transcription, translation, and processing of the IL-1α and IL-1β precursors, which will not be reviewed here. Two methods of controlling the action of mature IL-1 exist. One involves a dummy cytokine, IL-1Ra; the other a dummy receptor, the IL1R type II.

IL-1 receptor antagonist, or IL-1Ra [11, 12] is encoded in the same 400kb region of human chromosome 2 as IL-1α and IL-1β [13]. Its amino acid sequence is about as similar to those of IL-1α and IL-1β as those two sequences are to each other. The exon/intron structure is conserved in all three molecules as well, clearly indicating a common evolutionary origin [14]. All three bind to the IL1R-I with high affinity, with IL-1Ra having both the lowest Kd as well as the slowest off-rate. Nevertheless, IL-1Ra elicits no biological activity. This is a consequence of the inability of the IL-1Ra/IL1R-I complex to recruit AcP [8], unlike IL-1α- or IL-1β-bound IL1R-I. In the absence of the cytoplasmic heterodimer of the IL1R-I and AcP TIR domains, MyD88 is not recruited and no signaling takes place.

Recruitment of AcP is, in large part, determined by a single loop in the IL-1 protein fold. Alteration of the IL-1Ra amino acid residue aspartate 145 (lying within this loop) to lysine, the amino acid found at the same position in IL-1β, converts the antagonist to a partial agonist [15]. The three-dimensional structures of IL-1β

and IL-1Ra bound to IL-1R type I have been solved [16, 17], and the loop region containing Asp145 does not make contact with the IL-1R itself. It is not known how IL-1R AcP interacts with the IL-1/IL-1R complex. A model has been proposed in which AcP, rather than acting with IL1R type I like a pair of hands cupping IL-1 between them, instead wraps around the back side of the ligand-bound receptor, making only minimal contact with IL-1 [18]. Unfortunately, an experimentally-determined crystal structure of the tri-molecular complex (IL-1, IL-1R type I and IL-1R AcP) has not yet been produced.

Just as the antagonist IL-1Ra is evolutionarily related to the agonists IL-1α and IL-1β, the regulatory IL1R type II [19] is related to signaling IL-1R type I: their genes are adjacent on human chromosome 2, and they possess similar amino acid sequences and a conserved exon/intron structure [20]. Unlike the IL-1R type I, however, IL1R-II has no TIR domain; indeed, the cytoplasmic portion is only 29 amino acids long. The IL-1R type II has never been demonstrated to mediate any biological response to IL-1 [4]. Instead, by virtue of binding IL-1 but failing to signal, the IL-1R type II acts as a negative regulator of IL-1 actions, and has been termed a "decoy receptor" [21, 22].

While the type I receptor seems to be universally expressed, the IL-1R type II is made by a limited set of cell types: primarily B cells, monocytes, and neutrophils in the blood, and basal epithelium in tissues such as skin, vagina and urethra [23]. Interestingly, many of these cells are also good producers of IL-1, and one hypothesis to explain the development of two separate regulators of IL-1 action is that the type II receptor, on the surface of IL-1-producing cells, serves to limit the autocrine actions of IL-1. IL-1R type II is also found in a soluble form [24], released from cells *via* the actions of a metalloproteinase, probably TACE [25, 26]. The expression of both surface and soluble IL-1R type II is strongly up-regulated by anti-inflammatory agents, including IL-4 and glucocorticoids [27]. There is evidence that in some circumstances an alternatively spliced form of mRNA may also contribute to the production of soluble IL1R type II [28]. Naturally circulating levels of soluble IL-1R type II are in the range of 5-10ng/ml [29], although these can rise in certain chronic [30] or acute [31, 24] inflammatory settings.

The IL-1R type II binds to IL-1β with high affinity; binding to IL-1α and IL-1Ra is about two orders of magnitude less strong [29] (see Figure 1). The poor binding to IL-1Ra can be rationalized by the need for the two regulators of IL-1 not to bind to one another and negate each other's activity [32]. Poor binding to IL-1α is less easy to understand. There are data to suggest that most IL-1α is retained locally by the cells that make it, whereas IL-1β is the main form found systemically [33]. Thus, it has been suggested that the differential binding of IL-1α and IL-1β by IL-1R type II might allow the local actions of IL-1α in host defense, while controlling the greater pathological potential of systemically-circulating IL-1β.

The IL-1R accessory protein is also made in soluble as well as transmembrane forms [8, 34]. In this case however, the soluble form is generated from an alternatively spliced mRNA, which is present in most tissues at

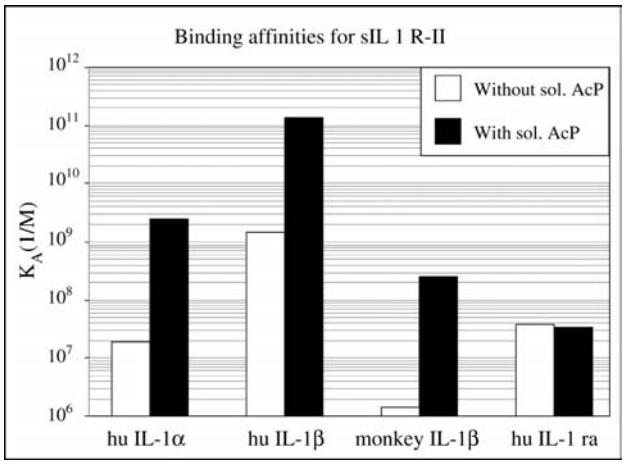

Figure 1
IL-1 receptor type II binding affinities. Binding affinities of human and monkey IL-1 ligands to human IL-1 receptor type II as measured by BIAcore. The affinity constants are expressed as 1/M and were determined either in the absence or presence of soluble AcP. See ref. [29] for details.

approximately 5-10% the level of the mRNA for full-length AcP [29]. Although studies have not been exhaustive, there is no evidence for more than modest regulation of AcP levels, either full-length or soluble [29, 34]. However, circulating levels of soluble AcP average over 300 ng/ml in humans, and are even higher in other animals (approximately 1 μg/ml in monkeys and up to 4 μg/ml in mice) [29]. These high levels immediately raise the question of what the function of soluble AcP might be.

One hypothesis for the function of soluble AcP is that it might associate with ligand-bound surface IL-1R type I, thus displacing full-length AcP from the complex and thereby preventing formation of the TIR domain heterodimer necessary for signaling. This was tested by Jensen *et al.* [34], who were unable to demonstrate an inhibitory function of "soluble" AcP unless they enhanced its surface concentration by tethering it to an unrelated membrane anchor. More recently, however, it has been reported that under certain conditions, soluble AcP can in fact inhibit IL-1 signaling in fibroblasts [35], by a mechanism which is as yet unknown.

Our group has recently published data demonstrating that soluble AcP can interact with ligand-bound soluble IL-1R type II, enhancing the latter's affinity for IL-1α and IL-1β by two orders of magnitude, while not affecting the very low affinity for IL-1Ra [29]. If this occurs *in vivo*, which is likely given the high circulating level of soluble AcP, then soluble IL-1R type II would become a very effective inhibitor, not only of IL-1β, but also of IL-1α.

It is not clear why nature should require cooperation between soluble AcP and soluble IL-1R type II in order for the latter to become a complete IL-1 inhibitor. It may be that it is simply a consequence of the way the membrane-bound forms of these receptors are designed to work. As for their soluble forms, the membrane form of the IL-1R type II can interact with the membrane form of AcP following ligand binding. This significantly enhances the affinity of binding [8], and for mouse IL-1R type II, is actually required for high-affinity binding of IL-1β [36, 37]. Indeed, increasing the expression of IL-1R type II decreases the responsiveness of a cell to

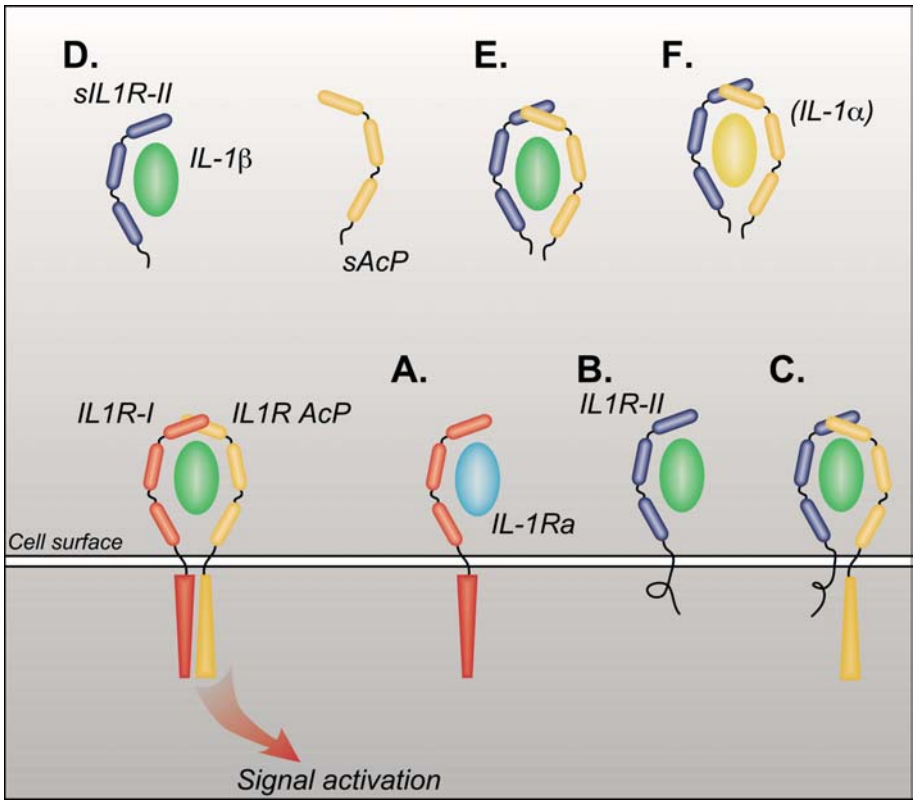

Figure 2

Regulation of mature IL-1 activity. This figure depicts the various regulatory complexes formed by IL-1 receptors and IL-1 ligands, compared to the active IL-1 signaling complex shown in the lower left. IL-1Ra binding to the IL-1 receptor type I (**A**) blocks receptor activation by IL-1α and IL-1β. Membrane-bound IL-1 receptor type II binds IL-1β with high affinity (**B**) and neutralizes its activity. This complex can also compete AcP away from productive signaling complexes (**C**). Shed IL-1 receptor type II maintains high neutralizing affinity for IL-1β in solution (**D**), but obtains even higher affinity due to cooperation with soluble AcP (**E**). Finally, the combination of soluble IL-1 receptor type II and soluble AcP is also able to bind IL-1α with high affinity (**F**).

IL-1, and at sufficiently high IL-1R type II expression, the cell becomes completely unresponsive to IL-1, presumably because all of the AcP is tied up in unproductive complexes with the IL-1R type II and is not available to interact with the signaling-competent IL-1R type I [38]. Thus, the ability of IL-1R type II to interact with AcP renders it a much more effective inhibitor of IL-1 than if it were simply a ligand sequestrant. It may be that the ability of IL1R-II to interact with AcP arose prior to the evolution of soluble forms of these proteins.

Interestingly, in mouse and monkey the dependence of the IL-1R type II on AcP for effective regulation of IL-1 action is even more pronounced than it is in human. Whereas human soluble IL-1R type II by itself binds human IL-1β with a Kd of approximately 10^{-9}M, a comparable level of affinity occurs in monkey only in the presence of soluble AcP [29] (see Figure 1). The difference is attributable to a single amino acid change between the human and monkey IL-1β molecules [39]. This change in human IL-1β, while not affecting its agonistic activity, renders it much more susceptible to neutralization by soluble IL1R-II. In mouse, although cooperation between surface IL-1R type II and surface AcP allows tight binding of IL-1 [37], we have been unable to demonstrate any binding of mouse IL-1β to soluble mouse IL-1R type II of soluble AcP (JES and DES, unpublished data).

Thus, the activity of mature IL-1 (both IL-1α and IL-1β) is regulated by a combination of an inactive ligand, IL-1Ra, and a decoy receptor, IL-1R type II (see Figure 2 for summary). There is evidence for the existence of a soluble form of the IL-1R type I as well [40], but it appears to be present at significantly lower levels and may not have physiological relevance. The ability of the IL-1R type II to inhibit is enhanced, in the case of human IL-1β, by the IL-1 receptor accessory protein, whereas for good inhibition of human IL-1α and of IL-1β in monkeys and mice, AcP is actually required. The level of circulating soluble AcP exceeds 300 ng/ml in humans, and is significantly higher in other species. High levels of soluble AcP will be helpful in driving binding of IL-1 to IL-1R type II to completion. There may be additional reasons for the high serum AcP level; however, the inability of AcP to bind IL-1, as well as the lack of significant regulation of its expression, would seem to preclude soluble AcP itself being a primary regulator of IL-1 action. Finally, it is of interest to note that in two instances of protein-protein interaction within the IL-1 system - recruitment of AcP by the IL-1R/IL-1Ra complex, and binding of monkey IL-1β to the IL-1R type II - alteration of a single amino acid results in a = 100-fold change in the affinity of the interaction. This would suggest that it may be possible to use small molecule drugs to modulate protein-protein interactions.

Regulation of the activity of mature IL-1 encompasses several complex mechanisms. Presumably, these have evolved because of the profound biological effects mediated by IL-1. It will be of interest in the future to learn in

which human diseases inhibition of IL-1 is of greatest benefit.

REFERENCES

1. Dinarello CA. 1998. Interleukin-1, interleukin-1 receptors and interleukin-1 receptor antagonist. (Review) *Int. Rev. Immunol.* 16: 457.
2. Konsman JP, Parnet P, Dantzer R. 2002. Cytokine-induced sickness behaviour: mechanisms and implications. (Review) *Trends Neurosci.* 25: 154.
3. Kolb M, Margetts PJ, Anthony DC, Pitossi F, Gauldie J. 2001. Transient expression of IL-1beta induces acute lung injury and chronic repair leading to pulmonary fibrosis. *J Clin Invest.* 107: 1529.
4. Sims JE, Gayle MA, Slack JL, Alderson MR, Bird TA, Giri JG, Colotta F, Re F, Mantovani A, Shanebeck K, *et al.* 1993. Interleukin 1 signaling occurs exclusively *via* the type I receptor. *Proc. Natl. Acad. Sci. USA.* 90: 6155.
5. Sims JE, March CJ, Cosman D, Widmer MB, MacDonald HR, McMahan CJ, Grubin CE, Wignall JM, Jackson JL, Call SM, *et al.* 1988. cDNA expression cloning of the IL-1 receptor, a member of the immunoglobulin superfamily. *Science.* 241: 585.
6. Sims JE, Acres RB, Grubin CE, McMahan CJ, Wignall JM, March CJ, Dower SK. 1989. Cloning the interleukin 1 receptor from human T cells. *Proc. Natl. Acad. Sci. USA.* 86: 8946.
7. Gay NJ, Keith FJ. 1991. Drosophila Toll and IL-1 receptor. *Nature.* 351: 355.
8. Greenfeder SA, Nunes P, Kwee L, Labow M, Chizzonite RA, Ju G. 1995. Molecular cloning and characterization of a second subunit of the interleukin 1 receptor complex. *J. Biol. Chem.* 270: 13757.
9. O'Neill LA. 2000. The interleukin-1 receptor/Toll-like receptor superfamily: signal transduction during inflammation and host defense. (Review) *Sci STKE.* 44:RE1.
10. Dayer JM, Bresnihan B. 2002. Targeting interleukin-1 in the treatment of rheumatoid arthritis. (Review) *Arthritis Rheum.* 46: 574.
11. Eisenberg SP, Evans RJ, Arend WP, Verderber E, Brewer MT, Hannum CH, Thompson RC. 1990. Primary structure and functional expression from complementary DNA of a human interleukin-1 receptor antagonist. *Nature.* 343: 341.
12. Hannum CH, Wilcox CJ, Arend WP, Joslin FG, Dripps DJ, Heimdal PL, Armes LG, Sommer A, Eisenberg SP, and Thompson RC. 1990. Interleukin-1 receptor antagonist activity of a human interleukin-1 inhibitor. *Nature.* 343: 336.
13. Nicklin MJ, Weith A, Duff GW 1994. A physical map of the region encompassing the human interleukin-1 alpha, interleukin-1 beta, and interleukin-1 receptor antagonist genes. *Genomics.* 19: 382.
14. Taylor SL, Renshaw BR, Garka KE, Smith DE, Sims JE. 2002. Genomic organization of the interleukin-1 locus. *Genomics.* 79: 726.
15. Ju G, Labriola-Tompkins E, Campen CA, Benjamin WR, Karas J, Plocinski J, Biondi D, Kaffka KL, Kilian PL, Eisenberg SP, *et al.* 1991. Conversion of the interleukin 1 receptor antagonist into an agonist by site-specific mutagenesis. *Proc. Natl. Acad. Sci. USA.* 88: 2658.
16. Vigers GP, Anderson LJ, Caffes P, Brandhuber BJ. 1997. Crystal structure of the type-I interleukin-1 receptor complexed with interleukin-1beta. *Nature.* 386: 190.
17. Schreuder H, Tardif C, Trump-Kallmeyer S, Soffientini A, Sarubbi E, Akeson A, Bowlin T, Yanofsky S, Barrett RW. 1997. A new cytokine-receptor binding mode revealed by the crystal structure of the IL-1 receptor with an antagonist. *Nature.* 386: 194.
18. Casadio R, Frigimelica E, Bossu P, Neumann D, Martin MU, Tagliabue A, Boraschi D. 2001. Model of interaction of the IL-1 receptor accessory protein IL-1RacP with the IL-1beta/IL-1R(I) complex. *FEBS Lett.* 499: 65.
19. McMahan CJ, Slack JL, Mosley B, Cosman D, Lupton SD, Brunton LL, Grubin CE, Wignall JM, Jenkins NA, Brannan CI, *et al.* 1991. A novel IL-1 receptor, cloned from B cells by mammalian expression, is expressed in many cell types. *EMBO J.* 10: 2821.
20. Sims JE, Painter SL, Gow IR. 1995. Genomic organization of the type I and type II IL-1 receptors. *Cytokine.* 7: 483.
21. Colotta F, Re F, Muzio M, Bertini R, Polentarutti N, Sironi M, Giri JG, Dower SK, Sims JE, Mantovani A. 1993. Interleukin-1 type II receptor: a decoy target for IL-1 that is regulated by IL-4. *Science.* 261: 472.
22. Colotta F, Dower SK, Sims JE, Mantovani A. 1994. The type II "decoy" receptor: a novel regulatory pathway for interleukin 1. (Review) *Immunol Today.* 15: 562.
23. Deyerle KL, Sims JE, Dower SK, and Bothwell MA. 1992. Pattern of IL-1 receptor gene expression suggests role in noninflammatory processes. *J. Immunol.* 149: 1657.
24. Giri JG, Wells J, Dower SK, McCall CE, Guzman RN, Slack J, Bird TA, Shanebeck K, Grabstein KH, Sims JE, *et al.* 1994. Elevated levels of shed type II IL-1 receptor in sepsis. Potential role for type II receptor in regulation of IL-1 responses. *J. Immunol.* 153: 5802.
25. Mullberg J, Rauch CT, Wolfson MF, Castner B, Fitzner JN, Otten-Evans C, Mohler KM, Cosman D, Black RA. 1997. Further evidence for a common mechanism for shedding of cell surface proteins. *FEBS Lett.* 401: 235.
26. Orlando S, Sironi M, Bianchi G, Drummond AH, Boraschi D, Yabes D, Mantovani A. 1997. Role of metalloproteases in the release of the IL-1 type II decoy receptor. *J. Biol. Chem.* 272: 31764.
27. Mantovani A, Locati M, Vecchi A, Sozzani S, Allavena P. 2001. Decoy receptors: a strategy to regulate inflammatory cytokines and chemokines. (Review) *Trends Immunol.* 22: 328.
28. Liu C, Hart RP, Liu XJ, Clevenger W, Maki RA, De Souza EB. 1996. Cloning and characterization of an alternatively processed human type II interleukin-1 receptor mRNA. *J. Biol. Chem.* 271: 20965.
29. Smith DE, Hanna R, Della Friend, Moore H, Chen H, Farese AM, MacVittie TJ, Virca GD, Sims JE. 2003. The soluble form of IL-1 receptor accessory protein enhances the ability of soluble type II IL-1 receptor to inhibit IL-1 action. *Immunity.* 18: 87.
30. Jouvenne P, Vannier E, Dinarello CA, Miossec P. 1998. Elevated levels of soluble interleukin-1 receptor type II and interleukin-1 receptor antagonist in patients with chronic arthritis: correlations with markers of inflammation and joint destruction. *Arthritis Rheum.* 41: 1083.
31. Pruitt JH, Welborn MB, Edwards PD, Harward TR, Seeger JW, Martin TD, Smith C, Kenney JA, Wesdorp RI, Meijer S, Cuesta MA, Abouhanze A, Copeland EM. 3rd, Giri J, Sims JE, Moldawer LL, Oldenburg HS. 1996. Increased soluble interleukin-1 type II receptor concentrations in postoperative patients and in patients with sepsis syndrome. *Blood.* 87: 3282.
32. Burger D, Chicheportiche R, Giri JG, Dayer JM. 1995. The inhibitory activity of human interleukin-1 receptor antagonist is enhanced by type II interleukin-1 soluble receptor and hindered by type I interleukin-1 soluble receptor. *J. Clin. Invest.* 96: 38.
33. Kurt-Jones EA, Beller DI, Mizel SB, Unanue ER. 1985. Identification of a membrane-associated interleukin 1 in macrophages. *Proc. Natl. Acad. Sci USA.* 82: 1204.
34. Jensen LE, Muzio M, Mantovani A, Whitehead AS. 2000. IL-1 signaling cascade in liver cells and the involvement of a soluble form of the IL-1 receptor accessory protein. *J. Immunol.* 164: 5277.

35. van de Loo FA, Smeets RL, Arntz OJ, Bennink MB, Martin M, Joosten LAB, van den Berg WB. 2002. *Arthritis Rheum.* 9: S586. (abstract).

36. Lang D, Knop J, Wesche H, Raffetseder U, Kurrle R, Boraschi D, Martin MU. 1998. The type II IL-1 receptor interacts with the IL-1 receptor accessory protein: a novel mechanism of regulation of IL-1 responsiveness. *J. Immunol.* 161: 6871.

37. Malinowsky D, Lundkvist J, Laye S, Bartfai T. 1998. Interleukin-1 receptor accessory protein interacts with the type II interleukin-1 receptor. *FEBS Lett.* 429: 299.

38. Re F, Sironi M, Muzio M, Matteucci C, Introna M, Orlando S, Penton-Rol G, Dower SK, Sims JE, Colotta F, Mantovani A. 1996. Inhibition of interleukin-1 responsiveness by type II receptor gene transfer: a surface « receptor » with anti-interleukin-1 function. *J. Exp. Med.* 183: 1841.

39. Smith DE, Ketchem RR, Moore H, Anderson Z, Renshaw BR, Friend DJ, Sims JE. 2002. A single amino acid difference between human and monkey interleukin (IL)-1beta dictates effective binding to soluble type II IL-1 receptor. *J. Biol. Chem.* 277: 47619.

40. Svenson M, Hansen MB, Heegaard P, Abell K, Bendtzen K. 1993. Specific binding of interleukin 1 (IL-1) beta and IL-1 receptor antagonist (IL-1Ra) to human serum. High-affinity binding of IL-1Ra to soluble IL-1 receptor type I. *Cytokine*. 1993 5: 427.