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STRUCTURE-FUNCTION RELATIONSHIP IN THE IL-1 FAMILY

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1. ABSTRACT

The interleukin 1 (IL-1) family is a group of related cytokines including two agonist proteins (IL-1α and IL-1β), each derived by enzymatic cleavage of precursor proteins (pro-IL-1α and pro-IL-1β), and three forms of an antagonist protein (IL-1ra, icIL-1raI, icIL-1raII). IL-1 plays a key role in the onset and development of the host reaction to invasion, being an important factor in the initiation of the inflammatory response and in the triggering of immune functiokeyns. Due to its pleiotropic activity and to the high potency of its inflammatory effects, IL-1 activity is tightly regulated in the body by a complex network of control systems. These include the presence of two types of inhibitors, the receptor antagonist IL-1ra and the second type of IL-1 receptor (IL-1R_{II}), which is a natural scavenger of IL-1. Furthermore, regulation of IL-1 activity is attained by a strict hierarchy of binding affinity of the two receptors (the activating IL-1R_I and the inhibitory IL-1R_{II}) for the various members of the IL-1 family. Additional levels of control are represented by the presence of soluble forms of both receptors and of immature pro-IL-1 forms with different characteristics of activity and receptor binding capacity. To clarify the features of reciprocal interaction among ligands and receptors, in the attempt to understand the rules regulating the IL-1 system and its effectiveness, a deep analysis of the relationship between structure and function in the proteins of the IL-1 family becomes of key importance. Information on this line has been provided by several groups mainly with studies of mutagenesis of IL-1α, IL-1β and IL-1ra in parallel with biological assays of activity.

In this review, a survey of the available data is provided, in order to construct a hypothetical model of the functional structure of IL-1 proteins as a basis for future therapeutic interventions based on genetic and protein engineering.

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2. INTRODUCTION

The importance of IL-1 in the initiation and maintenance of adequate response to invasion has been established quite clearly and reported in detail in several excellent review articles (1-9). In synthesis, IL-1 is a cytokine produced mainly by mononuclear phagocytes in response to a variety of stimuli (in particular microbial components, irritants, etc.), and it represents one of the first events of reaction of an organism to infectious or inflammatory agents. IL-1 produced at the site of invasion/inflammation then stimulates surrounding cells to synthesize and release chemokines (which can attract to the site PMN, monocytes and T lymphocytes), and other cytokines (e.g. IL-2, IL-4, IL-6), and activates cells in various organs. Thus, IL-1 initiates both non-specific defensive mechanisms (*e.g.* the inflammatory response) and it stimulates and amplifies specific immunological reactions. Due to the extremely high efficiency of its actions, IL-1 production and activity need to be tightly regulated within the organism. In fact, dysregulation of either production or activating effects of IL-1 can lead to persistence of inflammation and to anomalous immune responses. The causal involvement of IL-1 in chronic inflammatory diseases

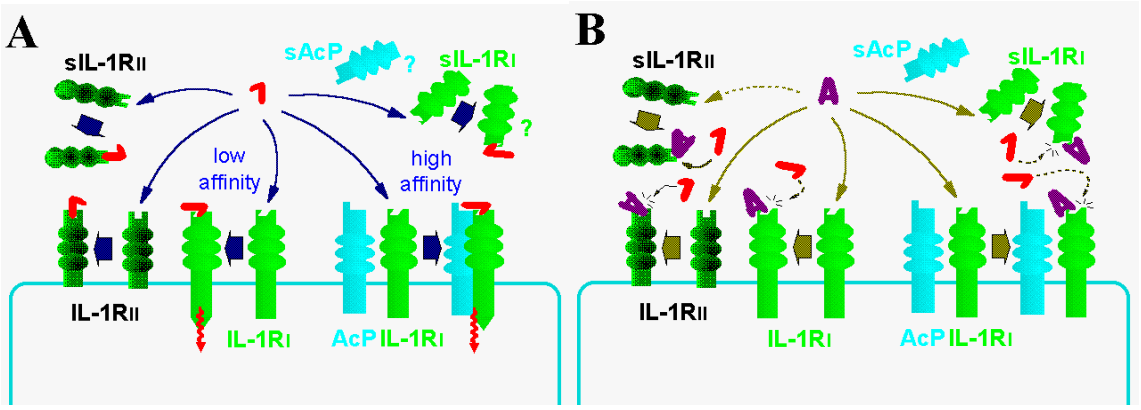

Figure 1: The IL-1 system

Panel A: Interaction of agonist IL-1α and IL-1β (represented as a red number one) with receptors on the surface of a responding cell is schematically represented in the cartoon. The agonist receptor IL-1R_I is represented in green color, the inhibitory receptor IL-1R_{II} in light green and the accessory protein IL-1RAcP in light blue. Soluble forms of the three receptors are also depicted. Initiation of intracellular signaling through the intracellular receptor domain is represented with a red arrow. The question mark indicates a biologically unknown or uncharacterized event.

Panel B: Interaction of antagonist IL-1ra (represented as a pink capital letter A) with receptors on the surface of an IL-1-responding cell is schematically represented in the cartoon. Symbols and colors are as in panel A. Interaction of IL-1ra with receptors prevents binding of agonist IL-1 and, in the case of IL-1R_I, association of IL-1RAcP. Binding of IL-1ra to soluble IL-1R_{II} is also represented, although its efficiency is very low and binding of IL-1β is preferred.

N-terminal sequences

1. secreted IL-1ra MEICRGLRSHLITLLLFLFHSETIC (leader peptide)

2. icIL-1raI MAL ETIC

3. icIL-1raII MAL ADLYEEGGGGGGEGEDNADSK ETIC

| exon 1 | exon 2 | exon 3 |

Common IL-1ra sequence

RPSGR KSSKM QAFRI WDVNQ KTFYL RNNQL VAGYL QGPNV NLEEK IDVVP IEPHA LFLGI HGGKM
CLSCV KSGDE TRLQL EAVNI TDLSE NRKQD KRFAF IRSDS GPTTS FESAA CPGWF LCTAM EADQP
VSLTN MPDEG VMVTK FYFQE DE

exons 3-6

Figure 2: Amino acid sequence of human IL-1ra proteins

Amino acid sequences of the three forms of human IL-1ra are shown. Differences in N-terminal sequences are indicated in correlation with the exons encoding them: exon 3 for secreted IL-1ra; exons 1 and 3 for icIL-1raI; exons 1, 2 and 3 for icIL-1raII. The common sequence, encoded by exons 3-6, corresponds to amino acids 1-152 of the mature secreted IL-1ra, after cleavage of the leader peptide. Amino acids are indicated with the one-letter code.

and in autoimmune syndromes has been indeed clearly established. To better understand the complex network of interactions governing the activity and the shut off of IL-1, the various components of the IL-1 system will be briefly described hereafter (Figure 1).

2.1 IL-1α, IL-1β and IL-1ra

The IL-1 family includes two agonist proteins, IL-1α and IL-1β, which are able to trigger cell activation upon interaction with specific receptors on the membrane of responding cells. Both proteins are synthesized as 31 kDa precursors (pro-IL-1α and pro-IL-1β) which are cleaved enzymatically to release the active mature 17 kDa IL-1 forms, i.e. the C-terminal fragments 113-271 for human IL-1α and 117-269 for human IL-1β. IL-1 proteins lack a signal peptide and thus are not secreted through classical pathways and are unglycosylated proteins. For IL-1α, a biologically active membrane-bound form has been described,

which is the myristoylated pro-IL-1α anchored to the membrane through lectin interaction with mannose residues. The preferential cell association of IL-1α, in contrast to the existence of IL-1β primarily as a soluble protein, leads to the hypothesis of different biological roles for the two types of IL-1. The third member of the IL-1 family is IL-1ra, a pure receptor antagonist, which is a glycosylated secretory protein of about 23 kDa, synthesized with a 25 amino acid-long signal peptide, subsequently cleaved to a mature protein of 152 amino acids.

Genes for IL-1α, IL-1β and IL-1ra are closely associated in the region 2q12-q21 of human chromosome 2, and are derived from gene duplications which occurred 320-400 M years ago (IL-1/IL-1ra divergence) and 270-300 M years ago (IL-1α/IL-1β duplication) (10, 11). IL-1 genes are composed of seven exons and six introns, with two untranslated regions located in exons 1-2 and in exon 7, respectively. Promoter regions of the IL-1α and IL-1β genes differ considerably, suggesting that the pattern of induction of the two genes in response to physiological and pathological stimulations are in fact distinct. The gene for IL-1ra presents a different organization, due to the fact that the same gene gives rise to three different protein products by alternative splicings (see amino acid sequences in Figure 2). The gene is composed by six exons and five introns. The secretory form of IL-1ra is coded for by the last four exons (exons 3-6), whereas the two intracellular forms of IL-1ra (icIL-1raI and icIL-1raII) are encoded by exons 1 + 3-6 and 1-6, respectively. The two intracellular forms of IL-1ra, present mainly in epithelial cells, are unglycosylated proteins which lack a signal peptide. These proteins, however, are able to exert biological activity which is identical to the functions of the secretory form of the protein (12, 13). Their physio-pathological role is still matter of debate.

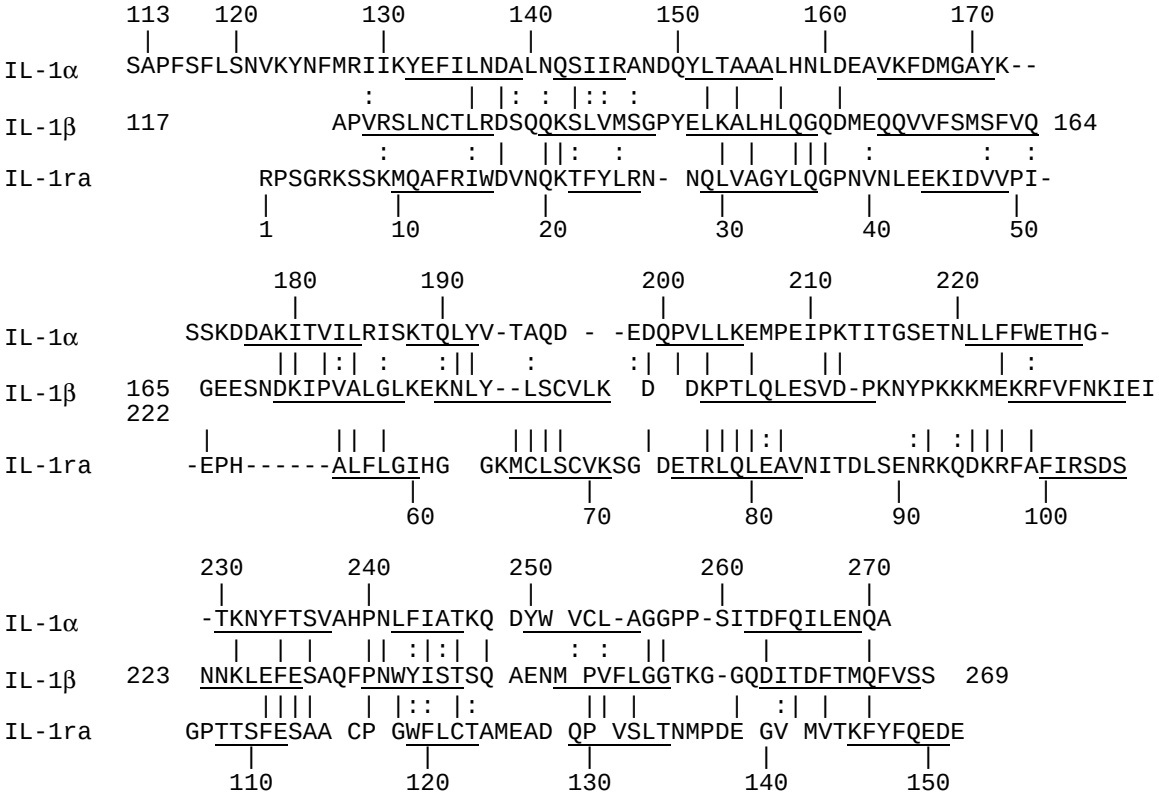

Figure 3: Sequence alignment of human IL-1 proteins

Alignment of amino acid sequences of the mature forms of human IL-1α, human IL-1α and human secretory IL-1ra is shown (14, 16, 18). Numbering refers to that of pro-IL-1 for IL-1α and IL-1β (with the first residue of mature IL-1α being number 113, and the first of mature IL-1β being number 117) and to that of the mature protein, after removal of leader, for IL-1ra. Identities are indicated by vertical bars, whereas homologies are indicated by dots. Residues forming the twelve β strands of the IL-1 structure are underlined.

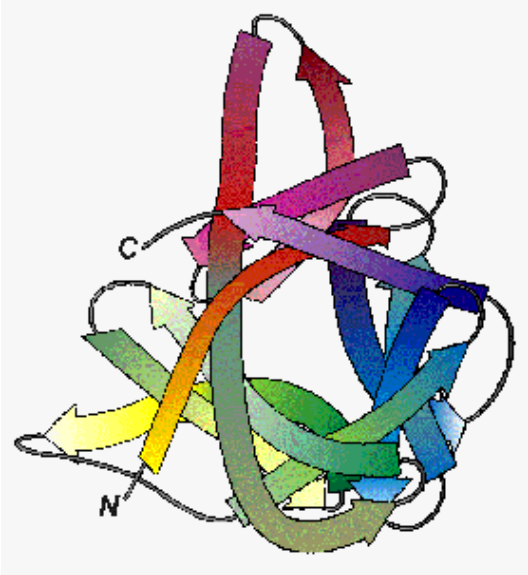

Figure 4: Structure of IL-1 proteins

The 3D structure of human mature IL-1β is represented in the cartoon, viewed down the axis of the β-barrel (14, 15, 119, 120). Structures of IL-1α and IL-1ra are very similar to that of IL-1β and are not depicted here (16, 18, 19, 132, 133). The twelve β strands forming the IL-1 structure are represented by colored ribbons, with the arrowhead toward the C-terminal end of the protein. Loop sequences between β strands are represented as gray strings. Artwork by Paolo Ruggiero.

Proteins of the IL-1 family exhibit about 20% homology at the amino acid level (Figure 3), but are very similar in their overall structure. Resolution of the crystal structure of IL-1β, IL-1α and IL-1ra has revealed that IL-1 proteins are β-barrels with a pseudo 3-fold axis, composed by 12 β-strands organized in three trefoil units of four antiparallel β-strands (Figure 4). Six of the strands form the barrel, whereas the other six form a sort of triangular array which closes the bottom of the barrel (14-19). The N- and C-terminals of the protein, and the exposed loops between β-strands 4-5 (loop D) and 8-9 (loop H) are located at the open end of the barrel.

2.2 ICE

The enzyme which converts pro-IL-1β to the active 17 kDa mature form has been identified, cloned and characterized (20-22). ICE (Interleukin 1-Converting Enzyme) is a cysteine protease synthesized as a 45 kDa inactive precursor and which requires two internal cleavages to form the active heterodimeric enzyme. ICE can selectively cleave pro-IL-1β between N116 and A117, thus forming the mature polypeptide 117-269. Other enzymes, however, can also non-specifically cleave pro-IL-1β at different sites (e.g. HIV protease after residue 94, elastase after residues 103 and 113 and granzyme A after residue 120), generating abnormal IL-1β

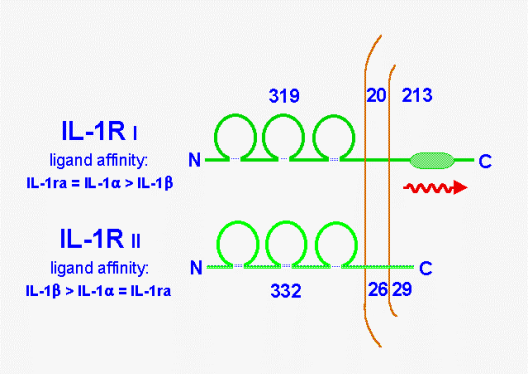

Figure 5: IL-1R$_I$ and IL-1R$_{II}$

The cartoon shows a schematic representation of the two types of IL-1R. Amino- and carboxyl-terminal ends of the receptors are indicated as N and C, respectively. The extracellular portion of receptors includes three Ig-like domains, represented as circles, with S-S bridges indicated as light lines. Numbers of residues of extracellular, transmembrane and intracellular domains are reported. In the intracellular domain of IL-1R$_I$, a thick area represents a potential protein kinase C acceptor site. The red arrow represents initiation of intracellular signaling. On the left is briefly reported the rank of ligand affinity (see text for detailed information).

molecules with reduced activity (23, 24). ICE is unable to convert pro-IL-1α to its mature form, but it can autocatalyze its own maturation. The homology of ICE with the protein encoded by the *Caenorhabditis elegans* gene *ced-3* (25) has led to a series of important observations and considerations. In *C. elegans*, *ced-3* is apparently responsible for programmed cell death, thus leading to the hypothesis that ICE plays a similar role in mammalian cells. Indeed, ICE and a series of newly discovered ICE-like enzymes appear to play a relevant role in cell apoptosis, independently of IL-1β maturation (26). The mechanisms of regulation of ICE activation in relation to both IL-1β production and cell death, and the reciprocal influence between the IL-1 system and the mechanisms of apoptosis, are currently matter of active investigation.

2.3 IL-1R$_I$, IL-1RAcP and signal transduction

Activation of target cells by IL-1 depends on the interaction of agonist proteins (IL-1α and IL-1β) with specific receptors on the cell membrane. Since the identification of specific receptors for IL-1, a wealth of information has been gathered on these structures (9, 27-30). Two types of IL-1R have been identified, IL-1R$_I$ and IL-1R$_{II}$ (Figure 4). These proteins are coded for by genes located in close proximity of the IL-1 genes on human chromosome 2 (31, 32). Of the two receptors, apparently only IL-1R$_I$ is able to trigger cell activation upon interaction with the agonist ligands.

IL-1 structure-function

IL-1R_I is a transmembrane protein belonging to the immunoglobulin superfamily, with a molecular weight of about 80 kDa (28, 9, 30). The extracellular domain of human IL-1R_I consists of 319 amino acids, encompasses three Ig-like domains and is glycosylated. This domain is responsible for ligand binding and interacts with similar affinity either with agonist proteins IL-1α and IL-1β or with the antagonist ligand IL-1ra. However, its interaction with IL-1β is less efficient than those with other ligands (Figure 5). In addition, IL-1R_I is able to bind with high affinity the pro-IL-1α molecule (which in fact is biologically active), whereas it cannot bind the pro-IL-1β protein (which does not posses any IL-1-like biological effect). IL-1R_I is expressed by all cells responsive to IL-1 and is the predominant type of IL-1R on T cells, fibroblasts, epithelial and endothelial cells.

The intracellular domain of IL-1R_I is responsible for the initiation of the signal transduction mechanism leading to cell activation. The sequential signal transduction pathway initiated by IL-1/IL-1R_I interaction has not been fully clarified, although a series of rapid events has been described which take place intracellularly within minutes after agonist binding. Among the events following receptor activation, hydrolysis of phospholipids, release of ceramide by neutral sphingomyelinase, multiple protein phosphorylation (including the IL-1R_I intracellular domain), activation of phosphatases, involvement of G proteins and GTP hydrolysis have been described (reviewed in 9, 33-35). These events are followed by release of lipid mediators, activation of MAP kinases, Ser/Thr kinases and of the so-called "IL-1R_I associated kinase". Some authors have described increase of cAMP, whereas no increase of intracellular Ca^{++} concentration could usually be detected. At later stages of the cell activation process, activation and nuclear translocation of transcriptional factors NFκB and AP-1 take place. A degree of difficulty in the understanding the pathways of IL-1-induced cell activation may come from the observation that in different cells different activation mechanisms can be used, involving different sets of events and enzymes. However, other explanations can be formulated. In fact, problems in understanding the mechanism of cell activation by IL-1 through IL-1R_I came by the common observation that responsiveness to IL-1 does not usually correlate with the number of IL-1R_I expressed on the cell surface, by identification of different classes of IL-1 binding affinity apparently attributable to IL-1R_I exclusively and by the notion that IL-1ra could interact with IL-1R_I without triggering cell activation.

These observations led to the hypothesis of the existence of a second receptor molecule which could complex to IL-1R_I and modulate intracellular signaling and cell activation. The recent identification of a receptor accessory protein (IL-1RAcP) will possibly fill several gaps in our knowledge of the

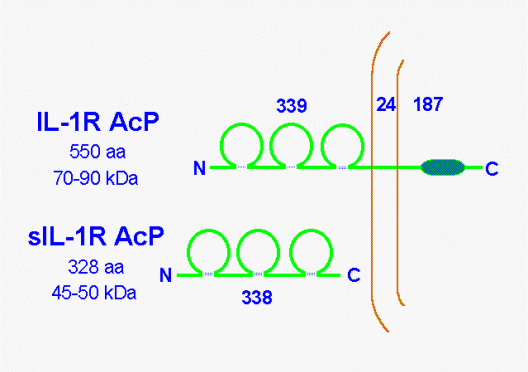

Figure 6: IL-1RAcP

The cartoon shows a schematic representation of the murine IL-1RAcP, both in its membrane (IL-1RAcP) and in its soluble (sIL-1RAcP) forms. Amino- and carboxyl-terminal ends of the receptors are indicated as N and C, respectively. The extracellular portion of IL-1RAcP includes three Ig-like domains, represented as circles, with S-S bridges indicated as light lines. Numbers of residues of extracellular, transmembrane and intracellular domains are reported. In the intracellular domain, a thick area represents a potential protein kinase C acceptor site.

IL-1R system (36). IL-1RAcP is structurally very similar to IL-1R_I, since it also belongs to the immunoglobulin superfamily, it is unable to bind IL-1 but it apparently associates to IL-1R_I to increase its binding affinity for agonist ligand IL-1α and IL-1β (Figure 6). Conversely, binding of the antagonist IL-1ra to IL-1R_I does not trigger association of IL-1RAcP and no signaling occurs. The precise role of IL-1RAcP in the signal transduction mechanisms triggered by IL-1 is however still a matter of investigation.

Both IL-1R_I and IL-1RAcP are also found in soluble form, encompassing the extracellular Ig-like domain of the protein. Whereas the soluble IL-1R_I apparently forms as result of a proteolytic event at the cell surface, soluble IL-1RAcP is the product of an alternative gene splicing. The functional role of these soluble receptors is not clear, in particular the role of sIL-1RAcP remains obscure. In the case of sIL-1R_I, its excellent binding capacity for IL-1ra might suggest a possible role in controlling the potency of IL-1 antagonism (37).

2.4 IL-1R_{II}

The second type of IL-1R, IL-1R_{II}, also belongs to the immunoglobulin superfamily and shares many structural characteristics of IL-1R_I. The main difference between IL-1R_{II} and IL-1R_I lies in the intracellular domain, which is extremely short for IL-1R_{II} and is apparently unable to initiate signal transduction (Figure 5). Thus, the functional role of IL-1R_{II} apparently is that of binding and sequestering IL-1, thus avoiding its interaction with IL-1R_I and

consequent cell triggering. IL-1R_{II} can therefore be considered as a natural inhibitor of IL-1 activity, a function complementary to that of IL-1ra (9, 30, 32, 38, 39). IL-1R_{II} is expressed by many cell types and is often co-expressed with IL-1R_I. It is particularly abundant on B cells, mononuclear phagocytes, polymorphonuclear leukocytes and bone marrow cells. Affinity of IL-1R_{II} for IL-1 proteins is quite different from what was observed for IL-1R_I. In fact, IL-1R_{II} can very efficiently bind IL-1β, whereas its affinity for IL-1α and IL-1ra is 10-100-fold lower. The extracellular IL-1-binding domain of IL-1R_{II} can be released from the cell surface by proteolytic cleavage and it can be found in biological fluids in large amounts during inflammatory conditions (40, 41). The biological significance of soluble IL-1R_{II} is still unclear. Indeed, sIL-1R_{II} maintains the same high affinity for IL-1β as the membrane IL-1R_{II} and thus has a pronounced inhibitory activity for IL-1β (39, 42). On the other hand, sIL-1R_{II} loses its ability to bind IL-1ra and acquires the capacity to bind pro-IL-1β (39). These observations can lead to the hypothesis that, in fact, sIL-1R_{II} may have a major role in the IL-1 inhibitory mechanisms, since it can capture active IL-1β with high affinity whereas it can not sequester the antagonist IL-1ra (37, 39). Furthermore, capture of inactive pro-IL-1β by sIL-1R_{II} prevents its maturation to the active mature form, thus contributing to the down-regulation of IL-1 effects (39).

2.5 Biological and pathological role

The biological effects of IL-1 are multiple and are directed at many cell types and organs (1-9). Briefly, the biological role of IL-1 could be defined as the initiation of the defense reaction. IL-1, and in particular IL-1β, are synthesized and released by mononuclear phagocytes and by many other cells in response to the classical inflammatory stimuli, such as microbial organisms and their components (2, 9). The activity of IL-1 in the inflammatory site initiates the host reaction to invasion. In fact, IL-1 stimulates the production of chemokines which can attract inflammatory cells such as PMN and monocytes, and immunocytes such as T lymphocytes (43). IL-1 can then activate the recruited cells to exert their defense functions (1-9). Furthermore, IL-1 can stimulate hematopoiesis by inducing synthesis of CSFs and by cooperating with them in the pathway of expansion and differentiation of myeloid precursors (9, 44, 45). *In vivo*, IL-1 shows a potent adjuvant effect and, in general, it enhances immune responses (46-48). IL-1 also induces strong inflammation, fever and pain (1-9, 49). Despite the fact that *in vitro* IL-1α apparently shares the vast majority of effects of IL-1β, its physiological role remains unclear. The fact that IL-1α is mostly found cell-associated, either membrane-bound or intracellularly as active pro-IL-1α or localized to the nucleus, has led to the hypothesis that this molecule has a predominant role in intracellular signaling and in cell-to-cell contact (9, 50-53).

Due to the potent inflammatory and immunostimulatory effects of IL-1β, its relevance in inflammatory and autoimmune pathologies has been investigated. Indeed, a large body of evidence has been gathered which indicates a causal role for IL-1β in a series of pathological derangements involving abnormal inflammatory and immune reactivity, in particular in the persistence of such conditions (9). Abnormal production or abnormal down-regulation of IL-1β has been demonstrated in septic shock, rheumatoid arthritis, inflammatory bowel diseases, psoriasis, autoimmune diabetes, bone and cartilage degradation in articular inflammation and osteoporosis, leukemias and solid tumors and many other pathological conditions (9).

Despite the fact that in several pathological situations the inhibition of IL-1 could be highly beneficial, the first trials with natural antagonists IL-1ra and sIL-1R_I did not provide exciting results (54). Thus, it becomes necessary to analyze in detail our knowledge of the IL-1 system in order to lay the bases for a rational anti-IL-1 therapy. The major difficulty in antagonizing IL-1 effects comes from the fact that optimal cell activation could be achieved by triggering of as little as ten receptors per cell, and that therefore an antagonist, to be effective, must counteract 100% of the available IL-1 (9). Furthermore, a complete and precise knowledge of agonist *vs.* antagonist interaction with the two types of receptor is still missing. In the following discussion, the information available to date on the relationship between structure and function in the proteins of the IL-1 family will be extensively reported, with particular attention to data coming from mutagenesis analysis, as necessary groundstone for the future rational construction of improved IL-1 inhibitors for therapeutic use.

3. DISCUSSION

Several studies have allowed in the last decade to clarify the structural characteristics of IL-1β, as well as of IL-1α and, more recently, of IL-1ra and to put them in relation with their capacity to bind to the specific receptors and to exert biological effects. Some of these investigations have taken advantage of specifically mapped monoclonal antibodies and of synthetic peptides in order to define, on the protein surface, sites selectively important for different activities or for receptor binding capacity (reviewed in 9, 55). As a general finding, from these studies the hypothesis can be drawn that different sites of the protein could be involved in distinct functions and that binding sites can be separated from active sites.

3.1 Mutagenesis

The major body of information on the structure-function relationship in proteins of the IL-1 family comes, however, from mutagenesis studies, in which the biological relevance of restricted areas of

the protein or even of single amino acids has been evaluated by residue substitution along the IL-1 sequence with techniques of genetic engineering (see also 9, 55). An extensive listing of single substitutions and multiple modifications, including deletions and insertions, is provided for human IL-1α (Tables I and II), for human IL-1β (Tables III and IV) and for human IL-1ra (Table V). As it can be noticed, the large majority of data that are available refer to IL-1β, rather than to IL-1α, because of the prominent role of this cytokine in the inflammatory events. Several observations can be made on these data. One important finding is that binding capacity for IL-1R_I does not correlate with binding to IL-1R_{II}, suggesting that despite the structural similarity between the two receptors the establishment of contact with receptors involves different domains of the IL-1 protein. Another general impression from observation of these data is that, although binding capacity for IL-1R_I is often associated to expression of biological activity, this is not always true. In fact, mutations at certain sites (*e.g.* R127 → G; 76, 82-87) do not significantly influence the IL-1R_I-binding capacity, but can deeply affect the agonist capacity, at least for some of the observed biological effects. In view of the recently discovered role of IL-1RAcP in the establishment of optimal agonist binding of IL-1 to IL-1R_I, this and other observations which dissociate binding to IL-1R_I from agonist effects should now be reconsidered. In fact, sites such as R127 can be selectively involved in the association of IL-1/IL-1R_I complex to IL-1RAcP and therefore important for biological activity eventhough irrelevant to IL-1R_I binding. Along the same line, it should also be considered that the importance of receptor binding for distinct biological effects appears to vary considerably. Thus, some activities apparently require involvement of IL-1RAcP whereas others may only need IL-1R_I binding. Ultimately, certain data of parallel mutagenesis studies of IL-1β and IL-1ra can help clarify the structural requirements for agonist *vs.* antagonist activity. For instance, the residue D261 of IL-1β, corresponding to the residue K145 of IL-1ra, is apparently pivotal for agonist capacity and possibly for interaction with IL-1RAcP. In fact, replacement of D261 with K in IL-1β does not affect binding but deeply impairs biological effects, whereas substitution of K145 with D in IL-1ra leaves binding unaltered but induces IL-1-like agonist capacity (56, 65, 82, 83, 98, 113). Another domain of high importance for agonist capacity of IL-1β is the β-bulge located between β strands 4 and 5 (loop D) which, however, is not involved in receptor binding and therefore can possibly take part in interaction with IL-1RAcP. In fact, insertion of this domain within the IL-1ra protein leads to acquisition of partial agonist activity without significant variation of receptor binding (113). This finding is indeed in line with previous reports showing selective IL-1-like biological activity by synthetic fragments corresponding to loop D in the absence of binding to IL-1R_I (55, 114-118). It is thus hypothesized that the loop D of IL-1β is responsible for interaction with IL-1RAcP and for the expression of immunostimulatory activity, whereas other areas of the molecule are more relevant to binding to IL-1R_I and to agonist inflammatory effects.

3.2 Structural analysis

Valuable information has been obtained from structural studies of IL-1 proteins, either from its crystallographic analysis or from NMR spectroscopy. Also in this case, analysis of IL-1β structure is far more detailed and complete than the information available for other proteins of the family. Crystallization of IL-1β and resolution of its 3D structure revealed a β-barrel structure with pseudo 3-fold symmetry, formed by twelve β-strands (14, 15, 119, 120). A large body of detailed structural information on IL-1β in solution has been provided by a series of excellent NMR studies, and by some studies with circular dichroism, fluorescence and calorimetry, which have allowed complete assignment of backbone and side chains, identification and position of associated water molecules and kinetics of folding and unfolding of the IL-1β protein (89, 121-130). Besides these studies on IL-1β, other important investigations regard structural analysis of IL-1β mutant proteins (81, 89, 94, 131), analysis of structural characteristics of IL-1α (16, 64) and of IL-1ra (18, 19, 132, 133). From all these data, a series of studies of computer-aided molecular modeling have been completed, which provide exhaustive information on the structural characteristics of agonist *vs.* antagonist IL-1 proteins in relation to their binding capacity and bioactivity (17, 72, 75, 83, 84, 96, 134, 135).

3.3 Models of ligand/receptor interaction

A summary of the models drawn from structural, conformational and biological data on IL-1 proteins could thus be provided. These models must, however, be proven true by experimental data, which are beginning to be available, on the crystallographic analysis of ligand/receptor complexes (136, 137). The available models also do not yet take into account the possible involvement of IL-1RAcP, which does not associate directly with IL-1 but which interacts with the IL-1/IL-1R_I complex. One common site of binding to IL-1R_I can be defined on agonist IL-1β and IL-1α and on antagonist IL-1ra. This area, defined as site A (83), located on the side of the β-barrel structure of IL-1 proteins, includes a discontinuous cluster of residues which include H146, Q131, Q148 and R127 in IL-1β (see also area II described in 75; and the hydrophilic binding area described in 96), N141 in IL-1α, and Y34, Q20, Q36, W16 and Y147 in IL-1ra (59, 75, 83, 85, 88, 96). Sites A of the three IL-1 proteins show remarkable structural similarity in molecular models and are considered to be responsible for the non-agonist binding with IL-1R_I. This site, in fact, represents the only contact site of IL-1ra for IL-1R_I, suggesting that interaction of IL-1 proteins with IL-1R_I through this area is not sufficient

for cell triggering. In the case of agonist proteins, IL-1α and IL-1β, another contact area for IL-1R_I has been identified which is missing in IL-1ra. This has been defined as site B and includes residues important for the biological activity of IL-1, i.e. R120, L122, F162, I172, K208, K209, K219, E221 in IL-1β (see also discontinuous binding site described in 72; and area I described in 75); R128, I130, D176, D177, K212, N220, W225, G229 and Q248 in IL-1α (58, 72, 75, 83). Comparison of structural models of IL-1 proteins shows that site B, located at the open end of the β-barrel, is present in the two agonist proteins, IL-1α and IL-1β, but absent in the IL-1ra structure, which in this particular area shows the highest degree of dissimilarity with other IL-1 molecules (15-18, 83, 113). Thus, site B is considered responsible for agonist binding to IL-1R_I. Initial crystallographic studies of the patterns of interaction between IL-1ra and IL-1R_I have shown that, in fact, the antagonist protein contacts the first and second Ig-like domain of IL-1R_I, whereas no strong interaction is established with the third domain (137). Modeling of IL-1β/IL-1R_I complex on the ground of the IL-1ra/IL-1R_I crystal analysis apparently indicates that the agonist IL-1 can interact, through a cluster of positively charged residues, with a negatively charged area in the third domain of IL-1R_I (137). In line with the identification of IL-1RAcP as a possibly important factor in determining agonist *vs.* non-agonist binding to IL-1R_I (IL-1RAcP can associate to IL-1α/IL-1R_I and IL-1β/IL-1R_I complexes but not to the IL-1ra/IL-1R_I complex; 36, 113). Thus, it can be proposed that, in the agonist action of IL-1, site A is responsible for the first contact with IL-1R_I, whereas site B takes part in a second type of interaction which involves the recruitment of IL-1RAcP into the complex. In IL-1ra, where only site A is present, no involvement of IL-1RAcP is possible and therefore no cell activation can be initiated.

3.4 Perspective

The large body of information available from mutagenesis and structural studies, together with those from studies with specific antibodies and with synthetic or recombinant peptides, has allowed to define the first general models of interaction of proteins of the IL-1 family with their receptors. The definition of agonist interaction of IL-1α and IL-1β with the activating receptor IL-1R_I will receive an enormous improvement from the recent identification of IL-1RAcP, the receptor accessory protein necessary for optimal interaction and receptor activation by agonist ligands. Further information will come from structural crystallographic analysis of IL-1/IL-1R complexes, to implement and clarify the present models.

Thus, it should be possible, within a short time, to unravel the precise features of agonist *vs.* non-agonist interactions of IL-1 proteins with their receptors, and to provide a solid background for the rational design of optimal antagonists for the therapeutic use. The study of interaction with the inhibitory receptor IL-1R_{II}, which is still scanty in comparison to data gathered for IL-1R_I, will also, in future, provide valuable information on the ways to control the IL-1 activities.

TABLE I

IL-1α:single amino acid substitutions

POSITION	AA CHANGE	BINDING TO IL-1R_I	BINDING TO IL-1R_{II}	ACTIVITY	REFS
113 (1)	S→A	n.t.	n.t.	↑1.5x (LAF, GIF) full (hE_2)	56, 57
125 (13)	N→A	↓2x (CHO)	↑.5x (70Z3)	n.t.	58
126 (14)	F→A	↓1.6x (CHO)	full (70Z3)	n.t.	58
127 (15)	M→A	↓2-3x (CHO)	↑3x (70Z3)	n.t.	58
	M→S	↓4-5x (CHO)	full (70Z3)	n.t.	58
128 (16)	R→A	↓125x (CHO); none (EL4)	↓6-10x (70Z3)	↓25x (D10)	58
	R→K	↓3x (CHO)	full (70Z3)	n.t.	58
130 (18)	I→A	↓2x (CHO); ↑3x (EL4)	↓1.5x (70Z3)	full (D10)	58
131 (19)	K→A	↓3x (CHO); full/↓1.6x (EL4)	full (70Z3)	full (D10)	58

132 (20)	Y→C	n.t.	n.t.	↓2x (LAF) full (GIF, hE_2)	56, 57
135 (23)	I→F	n.t.	n.t.	↓3x (LAF) full (GIF, hE_2)	56, 57
	I→L	n.t.	n.t.	full (LAF, GIF, hE_2)	56, 57
136 (24)	L→K	yes (EL4)	n.t.	↓100x (EL4 *vs.* bind.)	59
	L→S	yes (EL4)	n.t.	↓80-100x (EL4 *vs.* bind.)	59
	L→V	yes (EL4)	n.t.	↑20x (EL4 *vs.* bind.)	59
	L→W	yes (EL4)	n.t.	↑20x (EL4 *vs.* bind.)	59
137 (25)	N→S	n.t.	n.t.	↓2x (LAF) full (GIF, hE_2)	56
	N→R	yes (EL4)	n.t.	↑50x (EL4 *vs.* bind.)	59
138 (26)	D→V	n.t.	n.t.	↓10x (LAF) ↓1.5x (GIF) ↓50x (hE_2)	56, 57
	D→A	yes (EL4)	n.t.	↑25x (EL4 *vs.* bind.)	59
	D→C	yes (EL4)	n.t.	↑10x (EL4 *vs.* bind.)	59
	D→L	yes (EL4)	n.t.	↑30x (EL4 *vs.* bind.)	59
	D→P	yes (EL4)	n.t.	↑40x (EL4 *vs.* bind.)	59
	D→Q	yes (EL4)	n.t.	↑40x (EL4 *vs.* bind.)	59
	D→R	yes (EL4)	n.t.	↑10x (EL4 *vs.* bind.)	59
	D→S	yes (EL4)	n.t.	↑30x (EL4 *vs.* bind.)	59
	D→T	yes (EL4)	n.t.	↑50x (EL4 *vs.* bind.)	59
141 (29)	N→S	yes (EL4)	n.t.	full (LAF, GIF) ↑1.4x (hE_2) ↑20x (EL4 *vs.* bind.)	56, 57
	N→D	yes (EL4)	n.t.	↑50x (EL4 *vs.* bind.)	59
	N→L	yes (EL4)	n.t.	↑30x (EL4 *vs.* bind.)	59
	N→Y	yes (EL4)	n.t.	↑80x (EL4 *vs.* bind.)	59
142 (30)	Q→H	n.t.	n.t.	↑1.5x (D10)	60
	Q→R	n.t.	n.t.	↑1.4x (D10)	60
	Q→N	n.t.	n.t.	full (D10)	60
	Q→S	n.t.	n.t.	↓2x (D10)	60
	Q→C	n.t.	n.t.	↓2x (D10)	60
	Q→D	n.t.	n.t.	↓2.5x (D10)	60
	Q→Y	n.t.	n.t.	↓3x (D10)	60
	Q→G	n.t.	n.t.	↓5x (D10)	60
	Q→E	n.t.	n.t.	↓5x (D10)	60
	Q→L	n.t.	n.t.	↓7x (D10)	60
	Q→T	n.t.	n.t.	↓10x (D10)	60
	Q→I	n.t.	n.t.	↓200x (D10)	60
	Q→P	n.t.	n.t.	↓>1000x (D10)	60
146 (34)	R→K	n.t.	n.t.	full (D10)	60
	R→Q	n.t.	n.t.	full (D10)	60
	R→N	n.t.	n.t.	↓1.5x (D10)	60
	R→T	n.t.	n.t.	↓2x (D10)	60
	R→A	n.t.	n.t.	↓2x (D10)	60
	R→L	n.t.	n.t.	↓2x (D10)	60
	R→S	n.t.	n.t.	↓2x (D10)	60

	R→V	n.t.	n.t.	↓2x (D10)	60
	R→Y	n.t.	n.t.	↓2x (D10)	60
	R→C	n.t.	n.t.	↓2.5x (D10)	60
	R→F	n.t.	n.t.	↓2.5x (D10)	60
	R→P	n.t.	n.t.	↓2.5x (D10)	60
	R→I	n.t.	n.t.	↓2.5x (D10)	60
	R→G	n.t.	n.t.	↓5x (D10)	60
148 (36)	N→D	full (EL4)	n.t.	full (EL4, LAF, GIF, cyt, adj) ↓(pyr)	61-63
	N→S	full (EL4)	n.t.	full (EL4)	61
152 (40)	L→M	n.t.	n.t.	↓2x (D10)	60
	L→F	n.t.	n.t.	↓3x (D10)	60
	L→V	n.t.	n.t.	↓8x (D10)	60
	L→I	n.t.	n.t.	↓20x (D10)	60
	L→C	n.t.	n.t.	↓70x (D10)	60
	L→A	n.t.	n.t.	↓200x (D10)	60
	L→H	n.t.	n.t.	↓1000x (D10)	60
	L→R	n.t.	n.t.	↓>1000x (D10)	60
	L→N	n.t.	n.t.	↓>1000x (D10)	60
	L→Q	n.t.	n.t.	↓>1000x (D10)	60
	L→E	n.t.	n.t.	↓>1000x (D10)	60
	L→G	n.t.	n.t.	↓>1000x (D10)	60
	L→K	n.t.	n.t.	↓>1000x (D10)	60
	L→P	n.t.	n.t.	↓>1000x (D10)	60
	L→S	n.t.	n.t.	↓>1000x (D10)	60
	L→T	n.t.	n.t.	↓>1000x (D10)	60
153 (41)	T→A	n.t.	n.t.	↓2x (LAF) full (GIF, hE_2)	56, 57
158 (46)	H→A	full (EL4)	n.t.	n.t.	64
	H→R	full (EL4)	n.t.	n.t.	64
161 (49)	D→G	n.t.	n.t.	full (LAF, GIF, hE_2)	56
	D→A	↓2.5x (CHO)	↓2-2.5x (70Z3)	n.t.	58
162 (50)	E→G	n.t.	n.t.	full (LAF, GIF) ↓1.5x (hE_2)	56, 57
	E→V	n.t.	n.t.	full/↓1.5x (LAF, GIF) ↑1.8x (hE_2)	56, 57
168 (56)	M→L	n.t.	n.t.	full (LAF, hE_2) ↓1.6x (GIF)	56, 57
170 (58)	A→F	↓10x (CHO)	none (70Z3)	n.t.	58
171 (59)	Y→A	↓10-50x (CHO)	↓4-8x (70Z3)	n.t.	58
172 (60)	K→A	full (CHO)	full (70Z3)	n.t.	58
175 (63)	K→M	n.t.	n.t.	↓2.5x (LAF) full (GIF, hE_2)	56, 57
	K→R	n.t.	n.t.	↑1.4x (LAF) full (GIF, hE_2)	56, 57

	K→A	↑1.4-1.7x (CHO)	↑3.5x (70Z3)	n.t.	58
176 (64)	D→A	↓3-4x (CHO)	↑2x (70Z3)	n.t.	58
177 (65)	D→A	↓3-4x (CHO)	full (70Z3)	n.t.	58
	D→V	n.t.	n.t.	full (LAF, GIF, hE_2)	56, 57
179 (67)	K→A	↓1.3x/↑2.4x (CHO)	full (70Z3)	n.t.	58
180 (68)	I→A	↓25-50x (CHO) ↓3x (EL4)	full (70Z3)	↓10x (EL4)	58
181 (69)	T→A	n.t.	n.t.	↓2x (LAF, hE_2) full (GIF)	56, 57
	T→P	n.t.	n.t.	full (LAF, GIF, hE_2)	56, 57
	T→S	n.t.	n.t.	↓1.6x(LAF) full (GIF, hE_2)	56, 57
182 (70)	V→A	↓5-7x (CHO)	↓3x (70Z3)	n.t.	58
183 (71)	I→V	n.t.	n.t.	↑1.4x (LAF) full (GIF, hE_2)	56, 57
185 (73)	R→N	↓2x (CHO)	↓2x (70Z3)	n.t.	58
188 (76)	K→A	↓2x (CHO)	↓4x (70Z3)	n.t.	58
192 (80)	Y→A	↓4-5x (CHO)	↓3x (70Z3)	n.t.	58
194 (82)	T→A	n.t.	n.t.	full (LAF, hE_2) ↑1.5x (GIF)	56, 57
196 (84)	Q→A	full (CHO)	↓2x (70Z3)	n.t.	58
197 (85)	D→A	full (CHO)	↑2x (70Z3)	n.t.	58
198 (86)	E→A	full (CHO)	full (70Z3)	n.t.	58
199 (87)	D→V	n.t.	n.t.	↓2.5x (LAF) ↓1.5x (GIF) full (hE_2)	56, 57
	D→A	full (CHO)	↑1.5x (70Z3)	n.t.	58
205 (93)	K→R	n.t.	n.t.	full (LAF, GIF, hE_2)	56, 57
206 (94)	E→G	n.t.	n.t.	↑1.5-2x (LAF, hE_2) full (GIF)	56, 57
	E→V	n.t.	n.t.	full (LAF, hE_2) ↑1.5/↓2x (GIF)	56, 57
	E→K	full (CHO)	↑1.4x (70Z3)	n.t.	58
207 (95)	M→L	n.t.	n.t.	full (LAF, GIF, hE_2)	56, 57
209 (97)	E→A	full (CHO)	full (70Z3)	n.t.	58
210 (98)	I→V	n.t.	n.t.	↓1.5-2x (LAF, GIF) full (hE_2)	56, 57
	I→A	full (CHO)	full (70Z3)	n.t.	58

212 (100)	K→I	n.t.	n.t.	↓1.5-2x (LAF, GIF) ↑1.8x (hE_2)	56, 57
	K→R	n.t.	n.t.	full (LAF, hE_2) ↑1.5x (GIF)	56, 57
	K→A	↓5x (CHO)	full (70Z3)	n.t.	58
	K→D	↓7-20x (CHO) ↓10x (EL4)	↓1.5-3x (70Z3)	↓4x (D10)	58
213 (101)	T→A	↑1.6x (CHO)	full (70Z3)	n.t.	58
218 (106)	E→R	↓1.5x (CHO)	full (70Z3)	n.t.	58
220 (108)	N→A	yes (EL4) full/↑2x (CHO)	↑1.8x (70Z3)	↑80x (EL4 *vs.* bind.)	58, 59
	N→G	yes (EL4)	n.t.	↑100x (EL4 *vs.* bind.)	59
	N→I	yes (EL4)	n.t.	↑30x (EL4 *vs.* bind.)	59
	N→L	yes (EL4)	n.t.	↑30x (EL4 *vs.* bind.)	59
	N→M	yes (EL4)	n.t.	↑100x (EL4 *vs.* bind.)	59
	N→W	yes (EL4)	n.t.	↑25x (EL4 *vs.* bind.)	59
225 (113)	W→F	↓5-7x (CHO) ↓2.5x (EL4)	full (70Z3)	↓5-20x(D10)	58
227 (115)	T→A	↓2x (CHO); full (EL4)	↓2x(70Z3)	full (D10)	58
228 (116)	H→L	n.t.	n.t.	↑1.3x (LAF) full (GIF, hE_2)	56, 57
	H→R	n.t.	n.t.	↑1.4x (LAF) full (GIF) ↓4x (hE_2)	56, 57
229 (117)	G→P	yes (EL4)	n.t.	↑20x (EL4 *vs.* bind.)	59
	G→R	yes (EL4)	n.t.	↑15x (EL4 *vs.* bind.)	59
	G→V	yes (EL4)	n.t.	↑15x (EL4 *vs.* bind.)	59
231 (119)	K→M	n.t.	n.t.	↑1.7x (LAF) full (GIF, hE_2)	56, 57
235 (123)	T→A	n.t.	n.t.	full (LAF, GIF, hE_2)	56, 57
236 (124)	S→A	n.t.	n.t.	↓1.4-2x (LAF, GIF) ↑1.8x (hE_2)	56, 57
239 (127)	H→L	n.t.	n.t.	full (LAF) ↓1.5x (GIF) ↑1.8x (hE_2)	56, 57
	H→R	full (EL4)	n.t.	↑1.4-1.6x (LAF, hE_2) ↓1.4x (GIF)	56, 57, 64
	H→A	full (EL4)	n.t.	n.t.	64
241 (129)	N→D	n.t.	n.t.	↓1.4x (LAF) full (GIF, hE_2)	56, 57
	N→I	n.t.	n.t.	n.t.	56, 57
	N→S	n.t.	n.t.	↓20% (LAF) ↓2x (GIF) ↑1.4x (hE_2)	56, 57
	N→Y	n.t.	n.t.	full (LAF, GIF, hE_2)	56, 57

246 (134)	T→A	n.t.	n.t.	↓2x (LAF) full (GIF, hE_2)	56, 57
248 (136)	Q→L	n.t.	n.t.	[↓7x (LAF) ↓2x (GIF) ↑2x (hE_2)]	56, 57
	Q→C	yes (EL4)	n.t.	↓90x (EL4 *vs.* bind.)	59
	Q→E	yes (EL4)	n.t.	↑100x (EL4 *vs.* bind.)	59
249 (137)	D→A	n.t.	n.t.	↓2x (LAF) ↓1.3x (GIF) full (hE_2)	56, 57
	D→G	n.t.	n.t.	full (LAF) ↓2x (GIF) ↑1.5x (hE_2)	56, 57
	D→H	n.t.	n.t.	full (LAF) ↓1.3x (GIF) ↑1.5x (hE_2)	56, 57
	D→N	n.t.	n.t.	↑1.3-2x (LAF, GIF, hE_2)	56, 57
	D→Y	n.t.	n.t.	↓3x (LAF) ↑2x (GIF, hE_2)	56, 57
250 (138)	Y→C	n.t.	n.t.	↓1.3-1.6x (LAF, GIF) full (hE_2)	56, 57
251 (139)	W→F	full (EL4)	n.t.	n.t.	64
253 (141)	C→S	n.t.	n.t.	full (LAF, GIF, cyt, adj) ↓pyr	63
262 (150)	T→A	n.t.	n.t.	full (LAF, GIF, hE_2)	56, 57
263 (151)	D→G	n.t.	n.t.	↓1.5-4x (LAF, GIF, hE_2)	56, 57
	D→A	n.t.	n.t.	↓1.5-2.5x (LAF; hE_2) full (GIF)	56, 57
	D→E	n.t.	n.t.	↓1.6-2.5x (LAF; hE_2) full (GIF)	56, 57
	D→F	n.t.	n.t.	↓60-200x (LAF; GIF) none (hE_2)	56
	D→K	n.t.	n.t.	none (GIF, hE_2)	56
	D→R	n.t.	n.t.	none (GIF, hE_2)	56
	D→Y	↓1.5x (MG63)	n.t.	↓4-10x (LAF) ↓20-100x(GIF) ↓100x (IL-2) ↓1000x (h fibr prol) none (hE_2) ↓100x (rb E_2) ↓10x (rb pyr) ↓80x (rb ACTH *vt*) none (rb ACTH *vv*)	56, 57, 65
264 (152)	F→V	n.t.	n.t.	n.t.	56, 57
	F→N	yes (EL4)	n.t.	↓500x (EL4 *vs.* bind.)	59
	F→Q	yes (EL4)	n.t.	↓200x (EL4 *vs.* bind.)	59
	F→S	yes (EL4)	n.t.	↓500x (EL4 *vs.* bind.)	59

Legend to TABLE I

Position of residues is numbered from the pro-IL-1α sequence, thus the first amino acid of the mature IL-1α is 113 (in parentheses is indicated the numbering of mature sequence, to allow easy comparison with published data). Amino acids are indicated with the one-letter code.

Binding capacity and biological activities of mutant proteins are indicated in comparison to wild type IL-1α; *e.g.* "↓2x" stands for a two-fold decrease (i.e. 50%) *vs.* the wild type protein, whereas"↑2x"stands for a doubling of effect *vs.* that of wild type IL-1α.
n.t. = not tested; yes = activity present but not compared to that of the wild type protein; full = 100% of activity of the wild type protein; none = no activity detectable. Activities shown in brackets for mutant 248 Q→L refer to a very low protein yield, with consequent difficulty in obtaining reproducible results.

Binding to IL-1R_I is measured on different cell types, indicated in parentheses:
CHO = CHO cells transfected with hu r IL-1R_I;
EL4 = EL4-6.1 murine thymoma cells;
MG63 = human osteosarcoma line MG63.

Binding to IL-1R_{II} is measured on different cell types, indicated in parentheses:
70Z3 = murine B cell line 70Z3.

Biological activities are measured in different assays, indicated in parentheses:
LAF = murine thymocyte proliferation *in vitro*;
GIF = growth inhibition assay on A375 human melanoma cells *in vitro*;
hE_2 = induction of PGE_2 from human cells *in vitro*;
D10 = proliferation of the murine T cell clone D10.G4.1 *in vitro*;
EL4 = induction of IL-2 production in EL4 murine cells ("*vs.* binding" indicates that activity was not measured *vs.* the wild type protein but *vs.*
IL-1R_I binding capacity);
cyt = induction of cytokine (CSF, IFN, IL-2, IL-3) production *in vitro*;
adj = adjuvant effect *in vivo*;
pyr = pyrogenic effect *in vivo*;
IL-2 = induction of IL-2 production *in vitro*;
h fibr prol = proliferation of human fibroblasts;
rb E_2 = induction of PGE_2 from rabbit cells *in vitro*;
rb pyr = pyrogenicity in the rabbit *in vivo*;
rb ACTH *vt* = induction of ACTH production in rabbit cells *in vitro*;
rb ACTH *vv* = induction of ACTH production in the rabbit *in vivo*.

TABLE II

IL-1α: multiple modifications, deletions, insertions

POSITION	MODIFICATION	BINDING TO IL-1R_I	BINDING TO IL-1R_{II}	ACTIVITY	REFS
113-117	des-SAPFS	↓2x (EL4)	n.t.	↓3x (EL4)	66
113-118	des-SAPFSF	n.t.	n.t.	full (D10/1A5)	67
113-121	des-SAPFSFLSN	↓2x (EL4)	n.t.	↓1.7x (EL4)	66
113-125	des-SAPFSFLSNVKYNF	n.t.	n.t.	↓10x (D10/1A5)	67
113-126	des-SAPFSFLSNVKYNFM	↓3x (EL4)	n.t.	↓10x (EL4) yes (IL-6)	66, 68
113-128	des-SAPFSFLSNVKYNFMRI	↓>50x (EL4)	n.t.	↓200x (EL4)	66
113-129	des-SAPFSFLSNVKYNFMRII	↓>50x (EL4)	n.t.	↓200x (EL4)	66
113-130	des-SAPFSFLSNVKYNFMRIIK	↓>50x (EL4)	n.t.	↓6500x (EL4)	66
113-132	des-SAPFSFLSNVKYNFMRIIKYE	↓>50x (EL4)	n.t.	↓>6500x (EL4)	66
115-121	des-PFSFLSN	n.t.	n.t.	↓2x (D10)	60
115-123	des-PFSFLSNVK	n.t.	n.t.	↓2x (D10)	60

116-119	des-FSFL	n.t.	n.t.	full (D10)	60
118/119	des-F/L	n.t.	n.t.	↓2x (D10)	60
119/120	des-LS/ins-P	n.t.	n.t.	full (D10)	60
121	des-N	n.t.	n.t.	↓5x (D10)	60
125-128	NFMR→SWSR	n.t.	n.t.	yes (D10)	60
	NFMR→GLKG	n.t.	n.t.	yes (D10)	60
	NFMR→QGGR	n.t.	n.t.	yes (D10)	60
	NFMR→SFLR	n.t.	n.t.	yes (D10)	60
	NFMR→SLRI	n.t.	n.t.	yes (D10)	60
	NFMR→NAHG	n.t.	n.t.	yes (D10)	60
	NFMR→QLFK	n.t.	n.t.	yes (D10)	60
	NFMR→TNFR	n.t.	n.t.	yes (D10)	60
	NFMR→RQHA	n.t.	n.t.	yes (D10)	60
	NFMR→QRGR	n.t.	n.t.	yes (D10)	60
	NFMR→AHIK	n.t.	n.t.	yes (D10)	60
127/128	des-MR/ins-I	n.t.	n.t.	↓200x (D10)	60
129-132	IIKI→MHLR	n.t.	n.t.	yes (D10)	60
	IIKI→VIST	n.t.	n.t.	yes (D10)	60
	IIKI→WTQQ	n.t.	n.t.	yes (D10)	60
	IIKI→MLLH	n.t.	n.t.	yes (D10)	60
	IIKI→VWKQ	n.t.	n.t.	yes (D10)	60
	IIKI→VHKS	n.t.	n.t.	yes (D10)	60
	IIKI→LKKQ	n.t.	n.t.	yes (D10)	60
	IIKI→YVSQ	n.t.	n.t.	yes (D10)	60
	IIKI→IALS	n.t.	n.t.	yes (D10)	60
	IIKI→VLRG	n.t.	n.t.	yes (D10)	60
	IIKI→WKLY	n.t.	n.t.	yes (D10)	60
	IIKI→FTCQ	n.t.	n.t.	yes (D10)	60
	IIKI→TLCT	n.t.	n.t.	yes (D10)	60
	IIKI→VFLF	n.t.	n.t.	yes (D10)	60
133-136	EFIL→DAII	n.t.	n.t.	yes (D10)	60
	EFIL→EMAL	n.t.	n.t.	yes (D10)	60
	EFIL→GTIL	n.t.	n.t.	yes (D10)	60
	EFIL→EVLI	n.t.	n.t.	yes (D10)	60
	EFIL→RMVL	n.t.	n.t.	yes (D10)	60
	EFIL→AVSF	n.t.	n.t.	yes (D10)	60
	EFIL→SISL	n.t.	n.t.	yes (D10)	60
134/135	des-F/I	n.t.	n.t.	↓>1000x (D10)	60
134-136	des-F/I/L	n.t.	n.t.	↓>1000x (D10)	60
137/138	des-N/D	n.t.	n.t.	↓>1000x (D10)	60
137-141	NDALN→QDAAQ	n.t.	n.t.	yes (D10)	60
	NDALN→QASPA	n.t.	n.t.	yes (D10)	60
	NDALN→LPPNH	n.t.	n.t.	yes (D10)	60
	NDALN→FDALN	n.t.	n.t.	yes (D10)	60
	NDALN→TQSGY	n.t.	n.t.	yes (D10)	60
	NDALN→RTPWQ	n.t.	n.t.	yes (D10)	60
	NDALN→LNNRS	n.t.	n.t.	yes (D10)	60
	NDALN→KGAGF	n.t.	n.t.	yes (D10)	60
137/140/151	N/L/Y→D/M/D	yes (EL4)	n.t.	↓900x (EL4 *vs.* bind.)	59
138-140	des-DAL/ins-V	n.t.	n.t.	↓>1000x (D10)	60
138/140	D/L→E/W	yes (EL4)	n.t.	↓30x (EL4 *vs.* bind.)	59
138-141	des-DALN	n.t.	n.t.	↓>1000x (D10)	60
138/143	D/S→E/T	yes (EL4)	n.t.	↓20x (EL4 *vs.* bind.)	59
138/145/153	D/I/T→E/M/A	yes (EL4)	n.t.	↓70x (EL4 *vs.* bind.)	59
140	des-L	n.t.	n.t.	↓500x (D10)	60

142/143	des-Q/S	n.t.	n.t.	↓>1000x (D10)	60
142/144	Q/I→L/S	yes (EL4)	n.t.	↓80x (EL4 *vs.* bind.)	59
142-146	QSIIR→QRILR	n.t.	n.t.	yes (D10)	60
	QSIIR→RYLLK	n.t.	n.t.	yes (D10)	60
143-145	SII→SVM	n.t.	n.t.	yes (D10)	60
	SII→FVI	n.t.	n.t.	yes (D10)	60
	SII→LVY	n.t.	n.t.	yes (D10)	60
	SII→TVI	n.t.	n.t.	yes (D10)	60
	SII→IVQ	n.t.	n.t.	yes (D10)	60
144/145	des-I/I	n.t.	n.t.	↓>1000x (D10)	60
145/146/147/151	IRAY→MPSN	yes (EL4)	n.t.	↓600x (EL4 *vs.* bind.)	59
147-149	des-AND	n.t.	n.t.	↓>1000x (D10)	60
147-150	ANDQ→QNDR	n.t.	n.t.	yes (D10)	60
	ANDQ→DTAN	n.t.	n.t.	yes (D10)	60
	ANDQ→AHSR	n.t.	n.t.	yes (D10)	60
	ANDQ→HSPK	n.t.	n.t.	yes (D10)	60
	ANDQ→SPNK	n.t.	n.t.	yes (D10)	60
	ANDQ→QRTK	n.t.	n.t.	yes (D10)	60
	ANDQ→LHTN	n.t.	n.t.	yes (D10)	60
148/253	N/C→D/S	n.t.	n.t.	yes (IL-6) none (pyr)	68
151-154	YLTA→TLYA	n.t.	n.t.	yes (D10)	60
	YLTA→RLKP	n.t.	n.t.	yes (D10)	60
	YLTA→RLTQ	n.t.	n.t.	yes (D10)	60
	YLTA→RLRY	n.t.	n.t.	yes (D10)	60
151-157	YLTAAAL→KLLMAGW	n.t.	n.t.	yes (D10)	60
	YLTAAAL→LLAAGPM	n.t.	n.t.	yes (D10)	60
	YLTAAAL→LLSSVHV	n.t.	n.t.	yes (D10)	60
	YLTAAAL→RLISAPR	n.t.	n.t.	yes (D10)	60
	YLTAAAL→VRATWLA	n.t.	n.t.	yes (D10)	60
	YLTAAAL→VLLGRTV	n.t.	n.t.	yes (D10)	60
	YLTAAAL→RLTRRYL	n.t.	n.t.	yes (D10)	60
153/154	des-T/A	n.t.	n.t.	↓20x (D10)	60
155/156	des-A/A	n.t.	n.t.	↓10x (D10)	60
156	des-A	n.t.	n.t.	↓200x (D10)	60
158-162	HNLDE→STLDN	n.t.	n.t.	yes (D10)	60
	HNLDE→PNAQT	n.t.	n.t.	yes (D10)	60
	HNLDE→DGRNS	n.t.	n.t.	yes (D10)	60
	HNLDE→LTDDR	n.t.	n.t.	yes (D10)	60
	HNLDE→TRSSP	n.t.	n.t.	yes (D10)	60
	HNLDE→RTAND	n.t.	n.t.	yes (D10)	60
	HNLDE→PESFE	n.t.	n.t.	yes (D10)	60
	HNLDE→PANQS	n.t.	n.t.	yes (D10)	60
	HNLDE→NDVLG	n.t.	n.t.	yes (D10)	60
	HNLDE→GNRHQ	n.t.	n.t.	yes (D10)	60
	HNLDE→LEESE	n.t.	n.t.	yes (D10)	60
	HNLDE→PHTTT	n.t.	n.t.	yes (D10)	60
	HNLDE→DVTSH	n.t.	n.t.	yes (D10)	60
	HNLDE→SNRHS	n.t.	n.t.	yes (D10)	60
	HNLDE→PTEDP	n.t.	n.t.	yes (D10)	60
	HNLDE→VRMEP	n.t.	n.t.	yes (D10)	60
	HNLDE→LQPTQ	n.t.	n.t.	yes (D10)	60
	HNLDE→MDFSG	n.t.	n.t.	yes (D10)	60
	HNLDE→GGGSE	n.t.	n.t.	yes (D10)	60
	HNLDE→RGYYD	n.t.	n.t.	yes (D10)	60

	HNLDE→DSTHF	n.t.	n.t.	yes (D10)	60
161/162	des-D/E	n.t.	n.t.	↓20x (D10)	60
163-165	AVK→SLR	n.t.	n.t.	yes (D10)	60
	AVK→PVY	n.t.	n.t.	yes (D10)	60
	AVK→ASH	n.t.	n.t.	yes (D10)	60
	AVK→KTY	n.t.	n.t.	yes (D10)	60
	AVK→PIK	n.t.	n.t.	yes (D10)	60
	AVK→VPR	n.t.	n.t.	yes (D10)	60
	AVK→ITT	n.t.	n.t.	yes (D10)	60
	AVK→IAK	n.t.	n.t.	yes (D10)	60
176/177	D/D→A/A	↓50x (CHO)	full (70Z3)	n.t.	58
185-186	L-R→ins-RILEL (-L*RILEL*R-)	n.t.	n.t.	↓>10000x (D10/1A5)	67
214-217	des-ITGS	n.t.	n.t.	↓1000x (D10)	60
215-218	des-TGSE/ins-K	n.t.	n.t.	↓500x (D10)	60
216,00	des-G	n.t.	n.t.	full (D10)	60
216/217	des-GS	n.t.	n.t.	full (D10)	60
	des-GS/ins-A	n.t.	n.t.	full (D10)	60
218/220/224/233	E/N/F/Y→A/I/Y/H	yes (EL4)	n.t.	↑20x (EL4 *vs.* bind.)	59
220/226/233	N/E/Y→K/D/D	yes (EL4)	n.t.	↑30x (EL4 *vs.* bind.)	59
220/226/235	N/E/T→Y/K/P	yes (EL4)	n.t.	↑20x (EL4 *vs.* bind.)	59
220/229/233	N/G/Y→T/D/F	yes (EL4)	n.t.	↓90x (EL4 *vs.* bind.)	59
221/223/232/233	L/F/N/Y→P/S/K/F	yes (EL4)	n.t.	↓90x (EL4 *vs.* bind.)	59
221/228/229	L/H/G→R/Q/V	yes (EL4)	n.t.	↑40x (EL4 *vs.* bind.)	59
223/226/228	F/E/H→L/V/D	yes (EL4)	n.t.	↑20x (EL4 *vs.* bind.)	59
224/228/230	F/H/T→D/D/I	yes (EL4)	n.t.	↓60x (EL4 *vs.* bind.)	59
239/247/253	H/K/C→T/N/S	yes (EL4)	n.t.	↑30x (EL4 *vs.* bind.)	59
239/248	H/Q→Y/L	yes (EL4)	n.t.	↓90x (EL4 *vs.* bind.)	59
244/252	I/V→S/L	yes (EL4)	n.t.	↓80x (EL4 *vs.* bind.)	59
247/249	K/D→T/H	yes (EL4)	n.t.	↑50x (EL4 *vs.* bind.)	59
258/264/265	P/F/Q→L/V/H	yes (EL4)	n.t.	↓90x (EL4 *vs.* bind.)	59
259/261	P/I→S/S	yes (EL4)	n.t.	↑20x (EL4 *vs.* bind.)	59
260/261/266/271	S/I/I/A→W/L/K/A+K	yes (EL4)	n.t.	↑20x (EL4 *vs.* bind.)	59
263-271	des-DFQILENQA	↓>50x (EL4)	n.t.	↓>5000x (EL4)	66
264-271	des-FQILENQA	↓>50x (EL4)	n.t.	↓>5000x (EL4)	66
265-271	des-QILENQA	↓>50x (EL4)	n.t.	↓>5000x (EL4)	66
266-271	des-ILENQA	↓20x (EL4)	n.t.	↓370x (EL4)	66
267-271	des-LENQA	↓5x (EL4)	n.t.	↓6x (EL4)	66
268-271	des-ENQA	↓7x (EL4)	n.t.	↓2x (EL4)	66

Legend to TABLE II

Deletions are indicated with "des-" in front of the deleted sequence; insertions are indicated with "ins-" in front of the inserted sequence. Residue numbering is that of the pro-IL-1α, with amino acid 113 being the first of the mature sequence. Amino acids are indicated with the one-letter code.

Binding capacity and biological activities of mutant proteins are indicated in comparison to wild type IL-1α; *e.g.* "↓2x" stands for a two-fold decrease (i.e. 50%) *vs.* the wild type protein, whereas"↑2x"stands for a doubling of effect *vs.* that of wild type IL-1α.
n.t. = not tested; yes = activity present but not compared to that of the wild type protein; full = 100% of activity of the wild type protein; none = no activity detectable.

Binding to IL-1R_I is measured on different cell types, indicated in parentheses:
EL4 = EL4-6.1 murine cells;
CHO = CHO cells transfected with hu r IL-1R_I.

Binding to IL-1R_{II} is measured on different cell types, indicated in parentheses:
70Z3 = murine B cell line 70Z3.

Biological activities are measured in different assays, indicated in parentheses:
EL4 = induction of IL-2 production in EL4 murine cells *in vitro*("*vs.* binding" indicates that activity was not measured *vs.* the wild type protein but *vs.* IL-1R_I binding capacity);
D10 = proliferation of the murine T cell clone D10.G4.1 *in vitro*;
1A5 = conversion assay with murine LBRM33-1A5 T cell *in vitro*;
pyr = pyrogenic effect *in vivo*;
IL-6 = induction of IL-6 production *in vitro.*

TABLE III

IL-1β: single amino acid substitutions

POSITION	AA CHANGE	BINDING TO IL-1R_I	BINDING TO IL-1R_{II}	ACTIVITY	REFS
117 (1)	A→T	n.t.	n.t.	↑4x (LAF)	69
120 (4)	R→K	↓2x (3T3) ↓100x (EL4)	↑1.5x (70Z3)	full (GIF) ↓2x (EL4)	70-72
	R→Q	↓5x (3T3) ↓3x (EL4)	n.t.	↓3x (GIF) ↓20x (EL4) none (pyr) yes (IL-6)	68, 70-72
	R→G	↓30x (3T3)	n.t.	0/↓90x (pyr) ↓20x (GIF) full (ACTH) yes (IL-6)	68, 71, 73, 74
	R→A	↓>100x (EL4)	↑1.5x (70Z3)	n.t.	72
	R→P	↓10x (EL4)	full (RAJI)	↓8x (EL4)	39, 70
	R→V	↓4x (EL4)	n.t.	↓20x (EL4)	70
	R→S	↓40x (EL4) ↓2.5-10x (h fibr, A375)	↓1.7x (RAJI)	↓10-20x (LAF, EL4, mE_2) ↓3-5x (hE_2, hEC)	70, 75, 76
	R→Y	↓10x (EL4) full (h fibr, A375)	full (RAJI)	↓5x (LAF, mE_2) full (hE_2, hEC)	75, 76
	R→D	0 (3T3) ↓>100x (EL4)	↓>100x (70Z3)	↓7300x (pyr) ↓15-1000x (GIF) ↓12.5x (LAF) ↓27x (E_2) ↓1.4x (CSF) ↓1.6x (PFC*vtr*) full (PFC*vv*, PMNwRx) yes (IL-6)	68, 71-73
121 (5)	S→R	full/↑2x (EL4)	full (RAJI)	full (EL4)	39, 70
	S→G	↓2-3x (EL4)	↑2x (RAJI)	↓3x (EL4)	39, 70
	S→E	↓2.5x (EL4)	n.t.	↓3x (EL4)	70
122 (6)	L→A	↓>100x/↓2x (EL4)	↑1.4x (70Z3)	↓20x (EL4)	70, 72
123 (7)	N→G	↓4x (EL4)	n.t.	↓6x (EL4)	70
	N→C	↓4x (EL4)	n.t.	↓2x (EL4)	70
	N→Q	full (EL4)	full//↓4x (1H7)	n.t.	77, 78
124 (8)	C→S	n.t.	n.t.	↓40x (GIF, LAF) ↓4x (EL4)	70, 79
	C→G	↓2.5x (EL4)	full (RAJI)	↓6x (LAF)	80

	C→A	n.t.	n.t.	full (GIF, LAF)	79
125 (9)	T→G	full (EL4)	full (RAJI)	↓200x (EL4)	39, 70, 81
	T→L	↑2x (EL4)	n.t.	↓2x (EL4)	70
	T→W	↑1.5x (EL4)	n.t.	↓5x (EL4)	70
	T→Q	↓8x (EL4)	n.t.	↓200x (EL4)	70
	T→E	↓5-300x (EL4)	full (RAJI)	↓200x (EL4)	39, 70
126 (10)	L→N	↓>1000x (EL4)	n.t.	↓10000x (EL4)	70
	L→T	↓>1000x (EL4)	n.t.	↓500x (EL4)	70
	L→C	↓>1000x (EL4)	n.t.	↓150x (EL4)	70
	L→S	↓>1000x (EL4)	n.t.	↓1000x (EL4)	70
	L→A	↓>1000x (EL4)	n.t.	↓500x (EL4)	70
127 (11)	R→A	full, ↓2x (CHO)	n.t.	full (D10)	82-84
	R→Q	full (3T3)	n.t.	full (GIF)	71
	R→E	↓3x (3T3)	n.t.	↓2x (GIF) ↓5x (D10)	71, 84
	R→T	↓2x (EL4) ↓3x (chondr)	n.t.	↓3x (LAF)	80
	R→K	n.t.	n.t.	full (D10)	84
	R→W	n.t.	n.t.	full (D10)	84
	R→S	full (EL4) ↑1.5-3.5x (h fibr, A375)	↓1.4x (RAJI)	full	75, 76, Vosbeck unp.
	R→G	full (EL4) ↓2x (CHO) ↓10x (A375)	↑2x (1H7)	full (jun/fos, IL-6)full/↓(MMP) ↓1000x (GIF) ↓100-5000x (D10) ↓20x (LAF)	76, 82-87
131 (15)	Q→G	↓>100x (CHO)	n.t.	n.t.	83
	Q→H	full (CHO)	n.t.	n.t.	83
132 (16)	K→Q	↓1.7-3x (EL4, h fibr)	↓3x (RAJI)	n.t.	75
140 (24)	Y→F	full (EL4)	n.t.	full (LAF)	88, 89
141 (25)	E→K	full (EL4) ↓1.5x (h fibr, A375)	↓3x (RAJI)	n.t.	75
	E→A	full (h fibr)	n.t.	↓(GIF)	90
143 (27)	K→C	full (EL4)	n.t.	full (LAF)	88, 89
146 (30)	H→Q	↓2x (EL4)	n.t.	n.t.	88
	H→A	↓20x	n.t.	n.t.	82
	H→N	↓30x (EL4)	n.t.	↓10x (LAF)	88, 89
	H→G	↓20x	n.t.	n.t.	82
	H→R	↓20-100x (EL4) ↓100x (chondr)	↓100x (RAJI)	↓32x (LAF)	88, 89
	H→E	↓50x (CHO)	n.t.	n.t.	83
	H→S	↓10x (CHO)	n.t.	n.t.	83
148 (32)	Q→G	↓>100x (CHO)	n.t.	n.t.	83

150 (34)	Q→E	↓4x (EL4) ↓1.5x (h fibr) ↑1.6x (A375)	↓2.5x (RAJI)	n.t.	75, 76
160 (44)	M→S	↓100x (EL4)	↓>100x (70Z3)	n.t.	72
162 (46)	F→D	↓>100x (EL4)	↓2-5x (70Z3)	n.t.	72
	F→A	↓>100x (EL4)	↓2x (70Z3)	n.t.	72
	F→Y	full (EL4)	↑1.4x (70Z3)	n.t.	72
164 (48)	Q→E	↓2x (EL4) ↓2.5-10x (h fibr) full (A375)	full (RAJI)	n.t.	75, 76
166 (50)	E→A	full (EL4)	full (1H7)	full/↓2x (LAF) ↓3x (IL-6) full (MMP)	76, 87
167 (51)	E→V	full (EL4)	full (1H7)	full (LAF, IL-6, MMP)	87
	E→R	↑1.5-2x (EL4)	↑2.5x (70Z3)	n.t.	72
	E→K	full (EL4) ↓2.5-10x (h fibr, A375)	↓1.7x (RAJI)	full (LAF, mE_2) ↓2.5-3x (hE_2, hEC)	75, 76
168 (52)	S→A	full (EL4)	full (1H7)	↓2x (LAF, IL-6) full (MMP)	87
169 (53)	N→E	full (EL4)	↑1.4x (70Z3)	n.t.	72
170 (54)	D→R	full (EL4)	full (RAJI)	↓32x (LAF)	91
171 (55)	K→V	↑2x (EL4)	↑5x (1H7)	↓3x (IL-6) full (LAF, MMP)	87
	K→Q	full (EL4, h fibr)	full (RAJI)	n.t.	75
172 (56)	I→A	↓>100x (EL4)	↓2.5x (70Z3)	n.t.	72
	I→G	↓10x (CHO)	n.t.	n.t.	83
174 (58)	V→A	↓>100x (EL4)	↓>100x (70Z3)	n.t.	72
180 (64)	E→K	full (EL4) ↑1.3-1.8x (h fibr, A375)	↑1.4x (RAJI)	n.t.	75, 76
184 (68)	Y→F	full (EL4)	n.t.	full (LAF)	88, 89
187 (71)	C→S	full/↓250x (EL4) ↓2x (chondr) full (3T3)	↓2x (RAJI)	↓2000x/full (LAF) full (GIF) ↓60000x (chondr) yes (Rx, carr)	70, 80, 88, 92, 93
	C→A	n.t.	n.t.	full (GIF, LAF)	70
190 (74)	K→Q	full (A375)	n.t.	n.t.	76
191 (75)	D→G	full (EL4, h fibr)	full (RAJI)	n.t.	75
192 (76)	D→V	full (EL4)	↓2x (RAJI)	n.t.	39

195 (79)	T→A	full (EL4)	↓2x (RAJI)	n.t.	39
199 (83)	E→N	↓2x (EL4) full (h fibr)	full (RAJI)	n.t.	75
202 (86)	D→S	↑2x (A375)	n.t.	n.t.	75
204 (88)	K→G	full/↑1.8x (EL4)	↓3x (70Z3)	n.t.	70, 72
	K→V	full/↑1.4x (EL4)	↓2x (RAJI)	full (EL4)	39, 70
	K→L	↑2x (EL4)	n.t.	full (EL4)	70
	K→N	full (EL4) ↓1.3-2x (h fibr, A375)	full (RAJI)	n.t.	75, 76
205 (89)	N→F	full/↑1.5x (EL4)	↓1.5x (RAJI)	full (EL4)	39, 70
	N→R	↑2.2x (EL4)	n.t.	full (EL4)	70
	N→G	↑1.5x (EL4)	n.t.	full (EL4)	70
	N→C	↑1.6x (EL4)	n.t.	full (EL4)	70
206 (90)	Y→R	↓1.4x (EL4)	n.t.	↓2x (EL4)	70
	Y→V	↓2x (EL4)	n.t.	↓2x (EL4)	70
	Y→S	↓2x (EL4)	n.t.	↓2x (EL4)	70
	Y→L	↓2-3x (EL4)	↓2x (RAJI)	↓2.5x (EL4)	39, 70
	Y→F	full (EL4)	n.t.	full	88, 89
	Y→G	↓1.4x (EL4) full (chondr)	full (RAJI)	↓25x (LAF)	80
208 (92)	K→E	↓>100x (EL4)	↓90x (70Z3)	n.t.	72
	K→S	↓1.4-2x (EL4)	full (RAJI)	↓1.3x (EL4)	39, 70
	K→R	↑3-4.7x (EL4)	↑1.5-2x (RAJI)	↑3x (EL4)	39, 70
	K→G	↓10x (CHO)	n.t.	n.t.	83
	K→N	↓2.5x (EL4) ↓1.4-10x (h fibr)	full (RAJI)	n.t.	75
209 (93)	K→L	↓>100x (EL4) ↓300x (mrR_I)	full (70Z3)	↓(GIF, pyr) full (ACTH)	71, 72, 94
	K→D	↓>1000x (mrR_I)	n.t.	↓(GIF)	71, 72, 94
	K→A	↓>100x (EL4)	↓3.5x (70Z3)	n.t.	72
	K→F	↓>100x (EL4)	↓2x (70Z3)	n.t.	72
	K→S	↓>100x (EL4)	↓4x (70Z3)	n.t.	72
	K→E	↓>100x (EL4)	↓2x (70Z3)	n.t.	72
	K→M	↓4x (EL4) ↓100x (CHO)	↑2-3x (RAJI)	↓10x (EL4)	39, 70, 83
	K→R	full (EL4)	↑2-3.5x (RAJI)	full (EL4)	39, 70
	K→G	↓>100x (CHO)	n.t.	n.t.	83
	K→Q	↓20x (CHO)	n.t.	n.t.	83
210 (94)	K→A	↓2x (EL4)	↓10x (70Z3)	n.t.	72
	K→W	↓2-5x (EL4)	full (RAJI)	↓2x (EL4)	39, 70
	K→M	↓2.5x (EL4)	n.t.	↓2.5x (EL4)	70
	K→G	↓3.5x (EL4)	n.t.	↓5x (EL4)	70
	K→N	full (EL4) ↓2-5x (h fibr)	full (RAJI)	↓2x (LAF) full (mE_2) ↓1.7-3x (hE_2, hEC)	75

211 (95)	M→R	↓7x (EL4)	n.t.	↓14x (EL4)	70
212 (96)	E→Q	↓2-3x (EL4, chondr)	↓2-3x (RAJI)	↓16x (EL4)	80
	E→A	full/↑2x (EL4)	full (70Z3)	n.t.	72
	E→V	↓3x (EL4)	n.t.	↓1.4x (EL4)	70
	E→G	↓1.4-2x (EL4)	full/↓2x (RAJI)	↓5x (EL4)	39, 70
	E→N	↑1.2-1.7x (EL4, h fibr)	full (RAJI)	n.t.	75
213 (97)	K→L	↓2.5-10x (EL4)	full (70Z3)	n.t.	72
	K→T	↓3x (EL4)	n.t.	full (EL4)	70
	K→R	↓3x (EL4)	n.t.	full (EL4)	70
	K→G	↓4x (EL4)	n.t.	↓3x (EL4)	70
	K→V	↓5x (EL4)	n.t.	↓3x (EL4)	70
	K→Q	full (EL4, h fibr)	full (RAJI)	n.t.	75
214 (98)	R→L	↓2x (EL4)	↓10x (70Z3)	↓(GIF)	71, 72
	R→E	↓2x (EL4)	↓10x (70Z3)	n.t.	72
	R→Q	↓5x (A375)	n.t.	n.t.	76
219 (103)	K→Q	↓20-100x (EL4) ↓2x (rb ch) full (h fibr)	↓2x (RAJI)	↓500-1300x (LAF, rb chondr) ↓10x (hE_2, hEC)	75, 80
	K→S	↓>100x (EL4)	full (70Z3)	n.t.	72
221 (105)	E→S	↓>100x (EL4)	full (70Z3)	n.t.	72
	E→K	↓100x (EL4) ↓3-10x (h fibr)	↓1.7x (RAJI)	↓100x (LAF) ↓50x (mE_2) ↓3-4x (hE_2, hEC)	75, 95
	E→G	↓3x (CHO)	n.t.	n.t.	83
222 (106)	I→S	↓1.6x (EL4)	↓1.4x (1H7)	↓5x (IL-6) ↑3x (LAF)	87
224 (108)	N→A	↓2x (EL4)	full (70Z3)	n.t.	72
226 (110)	L→A	↓2x; ↑1.4-1.6x (EL4)	↑2x (70Z3)	↓	72, 96
227 (111)	E→N	full (EL4, h fibr)	↑1.8x (RAJI)	n.t.	75
229 (113)	E→N	↓2-3x (EL4) full (h fibr)	full (RAJI)	n.t.	75
233 (117)	F→S	full (A375)	n.t.	n.t.	76
234 (118)	P→S	full (EL4, h fibr, A375)	full (RAJI)	n.t.	75, 76
242 (126)	Q→E	full (EL4, h fibr, A375)	↑1.5x (RAJI)	n.t.	75, 76
244 (128)	E→K	↓20-50x (EL4, h fibr) ↓10x (A375)	↓10x (RAJI)	↓1000x (A375)	70, 76
254 (138)	K→C	full (EL4)	n.t.	full (LAF)	97
257 (141)	Q→E	full (EL4, h fibr, A375)	↓1.7x (RAJI)	n.t.	75, 76

261 (145)	D→K	full	n.t.	↓100x (LAF) ↓5x (GIF) 0 (E_2) ↓(+ IL-1 inhib.)	56, 82, 98
	D→G	full	n.t.	full	82
	D→Y	n.t.	n.t.	full (LAF) ↓2x (GIF) ↓10x (E_2)	56
	D→S	full (EL4, h fibr)	full (RAJI)	n.t.	75
262 (146)	F→L	↓1.6x	n.t.	none (GIF, LAF, E_2)	99
263 (147)	T→G	↓1.4x (CHO)	n.t.	n.t.	83
264 (148)	M→A	full ↑1.4-2x (EL4)	↑3x (70Z3)	n.t.	72
266 (150)	L→A	↓2x (EL4)	full (70Z3)	n.t.	72
267 (151)	V→D	↓5x (EL4) full (h fibr, A375)	↑1.4x (RAJI)	n.t.	75, 76

Legend to TABLE III

Position of residues is numbered from the pro-IL-1β sequence, thus the first amino acid of the mature IL-1β is 117 (in parentheses is indicated the numbering of mature sequence, to allow easy comparison with published data).Amino acids are indicated with the one-letter code.

Binding capacity and biological activities of mutant proteins are indicated in comparison to wild type IL-1β; *e.g.* "↓2x" stands for a two-fold decrease (i.e. 50%) *vs.* the wild type protein, whereas "↑2x"stands for a doubling of effect *vs.* that of wild type IL-1β.
n.t. = not tested; yes = activity present but not compared to that of the wild type protein; full = 100% of activity of the wild type protein; none = no activity detectable.

Binding to IL-$1R_I$ is measured on different cell types, indicated in parentheses:
3T3 = murine fibroblasts 3T3;
EL4 = EL4-6.1 murine cells;
h fibr = human normal fibroblasts;
A375 = human melanoma line A375;
CHO = CHO cells transfected with hu r IL-$1R_I$;
chondr = normal chondrocyes;
mrR_I = murine recombinant IL-$1R_I$;
rb ch = rabbit normal chondrocytes.

Binding to IL-$1R_{II}$ is measured on different cell types, indicated in parentheses:
70Z3 = murine B cell line 70Z3;
RAJI = human Burkitt's lymphoma line RAJI;
1H7 = 1H7 clone of the human B cell lymphoma RAJI.

Biological activities are measured in different assays, indicated in parentheses:
LAF = murine thymocyte proliferation *in vitro*;
GIF = growth inhibition assay on A375 human melanoma cells *in vitro*;
EL4 = induction of IL-2 production in EL4 murine cells *in vitro*;
pyr = pyrogenic effect *in vivo*;
IL-6 = induction of IL-6 production *in vitro*;
ACTH = induction of ACTH production;
mE_2 = induction of PGE_2 from murine cells *in vitro*;
hE_2= induction of PGE_2 from human cells *in vitro*;
hEC = activation of human endothelial cells *in vitro*;

CSF = induction of CSF production;
PFC*vtr* = stimulation of plaque forming cell (PFC) activity (i.e. antibody production) *in vitro*;
PFC*vv* = stimulation of plaque forming cell (PFC) activity (i.e. antibody production) *in vivo*;
PMNwRx = stimulation of PMN recruitment after whole body irradiation *in vivo*;
D10 = proliferation of the murine T cell clone D10.G4.1 *in vitro*;
jun/fos = induction of jun/fos *in vitro*;
MMP = stimulation of matrix metalloproteinase production *in vitro*;
rb chondr = activation of rabbit chondrocytes *in vitro*;
Rx = radioprotection *in vivo*;
carr = modulation of carrageenan-induced inflammation *in vivo*;
+ IL-1 inhib = acquisition of IL-1 inhibitory capacity.

TABLE IV

IL-1β: multiple modifications, deletions, insertions

POSITION	MODIFICATION	BINDING TO IL-1R_I	BINDING TO IL-1R_{II}	ACTIVITY	REFS
N-terminal	M	↓10x (EL4)	n.t.	↓4-6x (LAF) ↓20x (GIF)	100-102
117	des-A	n.t.	n.t.	↓3-4x (LAF)	100
117/118	A/P→T/M	↑2x (EL4) ↑2.5x (fibr)	full (RAJI)	↑7x (LAF) ↑4-7x (LAF) ↓3x (GM-CSF fibr)	69, 103, 104
117/118/120	A/P/R→T/M/E	↓300-500x (EL4) ↓1000x (fibr)	↓4x (RAJI)	↓700x (LAF) ↓500-5000x (LAF) ↓20,000x (GM-CSF fibr)	69, 103, 104
117-119	des-APV	↓5x (EL4) ↓2x (3T3)	n.t.	↓2x (EL4) ↓7x (GIF)	66, 71
117-119/267-269	des-APV/des-VSS	n.t.	n.t.	↓7x (GIF)	71
117/120	des-A/R→D	n.t.	n.t.	none (pyr, ACTH)	74
117-120	des-APVR	↓>30x (EL4)	n.t.	↓100x (EL4) ↓200x (GIF)	66, 71
117-121	des-APVRS	↓>30x (EL4)	n.t.	↓100x (EL4) ↓300x (GIF)	66, 71
117-122	des-APVRSL	↓>30x (EL4)	n.t.	↓1200x (EL4) ↓3000x (GIF) ↓10^3-10^4x (pyr) yes (ACTH)	66, 71, 105
117-123	des-APVRSLN	↓>30x (EL4)	n.t.	↓1600x (EL4)	66
117-125	des-APVRSLNCT	n.t.	n.t.	↓5000x (GIF)	69
117-126	des-APVRSLNCTL	↓>1000x (EL4)	n.t.	↓>10^6x (EL4)	106
117-127	des-APVRSLNCTLR	↓>30x (EL4)	n.t.	↓>10^4x (D10)	66
117-132	des-APVRSLNCTLRDSQQK	↓15x (EL4) ↓>1000x (EL4)	n.t.	↓>10^4x (D10) ↓>10^6x (EL4) some (D10)	106-108
117-132/198-269	des-APVRSLNCTLRDSQQK/ des-LESV...VSS	↓>1000x (EL4)	n.t.	↓10^5x (EL4)	66
117-132/199-269	des-APVRSLNCTLRDSQQK/ des-ESV...VSS	↓300x (EL4)	n.t.	↓>10^4x (D10) ↓>10^5x (EL4)	103, 104
117-135	des-APVRSLNCTLRDSQQKSLV	↓>30x/20x (EL4)	n.t.	↓>10^4-10^5x (D10, EL4, E_2)	66, 107, 109
117-135/198-269	des-APVRSLNCTLRDSQQKSLV/ des-LESV...VSS	n.t.	n.t.	some (D10)	108
120	des-R	↓100x (3T3)	n.t.	↓30x (GIF) none (pyr) yes (IL-6)	68, 71

123/164-171	N/QGEESNDK→Q/NYPKKKME	↓2x (EL4)	full (1H7)	full (D10, E_2)	77
123/203	N/P→Q/S	↑2x (EL4)	full (1H7)	n.t.	77
	N/P→Q/H	full (EL4)	full (1H7)	n.t.	77
123/205	N/N→Q/L	↓540x (EL4)	full (1H7)	n.t.	77
123/205-213	N/loop7→Q/loop12	↓30x (EL4)	↓1.5x (1H7)	n.t.	77
123/206	N/Y→Q/G	↓2.6x (EL4)	full (1H7)	n.t.	77
123/206/207	N/Y/P→Q/G/S	↓5x (EL4)	full (1H7)	n.t.	77
	N/Y/P→Q/G/T	↓1.5x (EL4)	↓3x (1H7)	n.t.	77
123/206/210	N/Y/K→Q/P/R	↑1.3 (EL4)	↑3x (1H7)	n.t.	77
123/207	N/P→Q/S	↓3x (EL4)	full (1H7)	n.t.	77
	N/P→Q/N	↓4x (EL4)	↑2x (1H7)	n.t.	77
	N/P→Q/T	full (EL4)	↓3x (1H7)	n.t.	77
123/207/209	N/P/K→Q/S/N	↓30x (EL4)	↑2x (1H7)	n.t.	77
123/208	N/K→Q/Q	↓1.5 (EL4)	↑3x (1H7)	n.t.	77
123/208/210	N/K/K→Q/S/S	↓3x (EL4)	↑2x (1H7)	n.t.	77
123/209	N/K→Q/N	↓30x (EL4)	↑2x (1H7)	n.t.	77
	N/K→Q/Q	↓15x (EL4)	↑2x (1H7)	n.t.	77
	N/K→Q/L	↓40x (EL4)	↑3x (1H7)	n.t.	77
123/235	N/N→Q/E	n.t.	n.t.	full (E_2)	78
123/235/237	N/N/Y→Q/E/T	full (EL4)	↓4x (1H7)	none (E_2) ↓500x (LAF) ↓50x (IL-6) full (pyr, E_2 hypoth)	78
123/236	N/W→Q/R	full (EL4)	↓4x (1H7) [full *vs.* N123/Q]	full (IL-6, E_2)	78
123/236/237	N/W/Y→Q/R/H	↓1.5x (EL4)	full (1H7) [↑3.5x *vs.* N123/Q]	full (E_2)	78
	N/W/Y→Q/R/W	n.t.	n.t.	full (E_2)	78
123/237	N/Y→Q/T	n.t.	n.t.	none (E_2)	78
	N/Y→Q/H	n.t.	n.t.	full (E_2)	78
	N/Y→Q/K	full (EL4)	↓2x (1H7) [↑2x *vs.* N123/Q]	none (E_2 SMC) full (E_2 fibr, pyr) ↓100x (LAF) ↓4x (IL-6)	78
	N/Y→Q/A	full (EL4)	↓7x (1H7) [↓1.5x *vs.* N123/Q]	none (E_2) ↓500x (LAF) ↓10x (IL-6) full (E_2 hypoth, pyr)	78
	N/Y→Q/F	n.t.	n.t.	full (E_2)	78
	N/Y→Q/G	full (EL4)	↓2x (1H7) [↑1.5x *vs.* N123/Q]	none (E_2) ↓>1000x (LAF) ↓>50x (IL-6) full (E_2 hypoth, pyr)	78
	N/Y→Q/L	↑1.7x (EL4)	↓2x (1H7) [↑1.8x *vs.* N123/Q]	↓5-10x (E_2) ↓5x(LAF) full (IL-6)	78
	N/Y→Q/W	↓1.3x (EL4)	n.t.	full (IL-6, E_2)	78
124	des-C	n.t.	n.t.	↓30x (GIF, LAF)	79, 93, 96
124/187	C/C→S/S	good (3T3)	n.t.	full (GIF, LAF)	92, 93
	C/C→A/V	good (3T3)	n.t.	full (GIF, LAF)	92, 93

	C/C→A/S	good (3T3)	n.t.	full (GIF, LAF)	79, 92, 93
	C/C→A/A	good (3T3)	n.t.	full (GIF, LAF)	79, 92, 93
	C/C→CM-C/CM-C	n.t.	n.t.	↓2x (EL4, LAF)	110
125/127/263/265	T/R/T/Q→R/W/Y/E	↑5x (EL4)	↑1.5x (1H7)	full (LAF) ↓5x (IL-6)	87
127/263	R/T→W/Y	↑4x (EL4)	↓1.5x (1H7)	↓2-5x (LAF, IL-6)	87
141-269	des-ELK...VSS	↓>30x (EL4)	n.t.	↓>5000x (EL4)	66
150/151	Q/D→A/A	↓2-3x (EL4, chondr)	↓2x (RAJI)	↓3x (LAF)	80
157/267	V/V→K/D	↓2.5x (EL4)	↓1.5x (1H7)	↓2-5x (LAF, IL-6)	87
166/167	des-E/E	↓15x (EL4) full (chondr)	full (RAJI)	↓40x (LAF)	80, 111
168/169/170	des-S/N/D	↓10x (EL4)	full (RAJI)	↓1000x (LAF)	91
179/181	K/K→S/S	↓50x (EL4) ↓5x (chondr)	↓15x (RAJI)	↓6000x (LAF) ↓$5x10^4$x (chondr)	80, 111
187	des-C	n.t.	n.t.	↓1000x (GIF, LAF)	79, 96
191/192	D/D→K/K	↓1.3-4x (EL4, chondr)	n.t.	↓5x (LAF)	80
	D/D→A/A	↓1.3-2x (EL4, chondr)	n.t.	↓5x (LAF)	80
198-269	des-LES...VSS	↓>30x (EL4)	n.t.	↓>10^4-10^5x (EL4, D10, GIF)	66, 71, 106, 107
199/202	E/D→Q/N	↓2-2.5x (EL4, chondr)	↓2x (RAJI)	↓3x (LAF)	80
202/204/205	D/K/N→N/D/L	↓9x (EL4)	↓4x (1H7b)	↑5x (D10)	87
202/204/206/207/208	D/K/Y/P/K→N/D/S/E/N	↓550x (EL4)	↓3x (1H7b)	↓3x (D10)	87
204/208	K/K→S/S	↓3-6x (EL4, chondr)	↓3x (RAJI)	↓5x (LAF)	80
208/209/210	des-K/K/K	↓100x (EL4)	↓500x (RAJI)	↓1600x (LAF)	80
208/209/210/219 *22%/27%/18%/100%*	K/K/K/K→S-DABITC-K_S (1h)	↓10x (EL4) [↓1000x 6 h]	n.t.	↓10x (EC) [↓1000x6 h]	95
214	des-R	↓500x (mrR_I)	n.t.	↓(GIF) none (pyr) yes (IL-6)	68, 71, 94
219-269	des-KIE...VSS	n.t.	n.t.	↓>10^4x (GIF)	71
222/227	I/E→S/S	↑6x (EL4)	full (1H7)	full (LAF) ↓2x (IL-6)	87
225/261	K/D→T/K	full (EL4)	↑2x (1H7)	full (LAF, IL-6)	87
236/237	W/Y→F/F	full (EL4)	n.t.	full (LAF)	88, 89
237-269	des-YIS...VSS	n.t.	n.t.	↓1500x (GIF)	71
242/244	Q/E→A/A	↓3x (EL4) full (chondr)	full (RAJI)	↓60x (LAF) ↓6000x (chondr)	80, 111
253-269	des-TKGGQDITDFTMQFVSS	↓>1000x (EL4)	n.t.	↓>10^6x (EL4)	106
254/255/256	des-K/E/G	↓25x (EL4) ↓250x (chondr)	↓250x (RAJI)	↓3000x (LAF)	80
257/258	Q/D→V/M	↑1.4x (EL4)	n.t.	↓3-5x (LAF, IL-6)	87
257-269	des-QDITDFTMQFVSS	n.t.	n.t.	↓>1500x (GIF)	71

260/261	T/D→A/N	↓10x (EL4) ↓3x (chondr)	↓3x (RAJI)	↓20x (LAF)	80
260-269	des-TDFTMQFVSS	↓>30x (EL4)	n.t.	↓>5000x (EL4)	66
261-269	des-DFTMQFVSS	n.t.	n.t.	↓500x (GIF)	71
263-269	des-TMQFVSS	↓>30x (EL4)	n.t.	↓>5000x (EL4)	66
264-269	des-MQFVSS	↓10x (EL4)	n.t.	↓200x (EL4)	66
265-269	des-QFVSS	↓3x (EL4) ↓10x (3T3)	n.t.	↓35x (EL4) ↓300x (GIF) none (pyr)	66, 71, 112
266-269	des-FVSS	↓4x (EL4) ↓10x (3T3)	n.t.	↓15x (EL4) ↓30x (GIF)	66, 71
267-269	des-VSS	↓2-3x (EL4, 3T3)	n.t.	full (EL4) ↓3x (GIF)	66, 71
269	des-S	n.t.	n.t.	full (GIF)	71

Legend to TABLE IV

Residue numbering refers to the pro-IL-1β sequence, with amino acid 117 being the first residue of the mature IL-1β protein. Modification at residues 208/209/210/219 is reported as percent of change at each position in 1 h.For some multiple mutants, binding capacity is also reported in comparison to the single mutant N123→Q (mutation to avoid glycosylation in the yeast expression system, which however influences negatively the IL-1R_{II}-binding capacity).

Binding capacity and biological activities of mutant proteins are indicated in comparison to wild type IL-1β e.g. "↓2x" stands for a two-fold decrease (i.e. 50%) *vs.* the wild type protein, whereas "↑2x" stands for a doubling of effect *vs.* that of the wild type IL-1β.
n.t. = not tested
yes = activity present but not compared to that of the wild type protein
some = activity lower than that of the wild type protein but not precisely quantitated
full = 100% of activity as compared to the wild type protein
none = no activity detectable.

Binding to IL-1R_I is measured on different cell types, indicated in parentheses:
EL4 = EL4-6.1 murine cells

fibr = normal fibroblasts;
3T3 = murine fibroblasts 3T3

chondr = normal chondrocytes

mrR_I = murine recombinant IL-1R_I.

Binding to IL-1R_{II} is measured on different cell types, indicated in parentheses:
RAJI = human Burkitt's lymphoma line RAJI

1H7 = 1H7 clone of the human B cell lymphoma RAJI.

Biological activities are measured in different assays, indicated in parentheses:
LAF = murine thymocyte proliferation *in vitro*

GIF = growth inhibition assay on A375 human melanoma cells *in vitro*;
GM-CSF fibr = induction of GM-CSF production in fibroblasts *in vitro*;
EL4 = induction of IL-2 production in EL4 murine cells *in vitro*;
pyr = pyrogenic effect *in vivo*;
ACTH = induction of ACTH production;
D10 = proliferation of the murine T cell clone D10.G4.1 *in vitro*;
IL-6 = induction of IL-6 production *in vitro*;
E_2 hypoth = induction of PGE_2 from hypothalamic cells *in vitro*;
E_2 SMC = induction of PGE_2 from smooth muscle cells *in vitro*;
E_2 fibr = induction of PGE_2 from fibroblasts *in vitro*;

chondr = activation of chondrocytes *in vitro*;
EC = activation of endothelial cells *in vitro*

TABLE V

IL-1ra: single and multiple modifications, deletions, insertions

POSITION	MODIFICATION	BINDING TO IL-1R_I	BINDING TO IL-1R_{II}	INHIBITORY ACTIVITY	AGONIST ACTIVITY	REFS
Single mutations						
9	K→R	full	n.t.	full	n.t.	Ruggiero unpubl.
14	R→G	full (EL4)	n.t.	↓1.7x (EL4)	n.t.	83
16	W→G	↓4-6x (EL4) ↓10x (CHO)	n.t.	↓	no	82, 83
	W→R	↓5x (CHO)	n.t.	↓	no	82, 83
	W→K	↓>100x (CHO)	n.t.	n.t.	n.t.	83
	W→M	↓20x (CHO)	n.t.	n.t.	n.t.	83
	W→Q	↓5-10x (CHO)	n.t.	n.t.	n.t.	83
	W→Y	↓1.5x (CHO)	n.t.	n.t.	n.t.	83
18	V→G	full (EL4)	n.t.	n.t.	n.t.	83
19	N→A	↓1.6x (EL4)	n.t.	n.t.	n.t.	83
20	Q→G	↓100x (EL4)	n.t.	n.t.	n.t.	83
	Q→A	↓20x (CHO)	n.t.	n.t.	n.t.	83
	Q→Y	↓>100x (CHO)	n.t.	n.t.	n.t.	83
	Q→D	↓>100x (CHO)	n.t.	n.t.	n.t.	83
	Q→K	↓>100x (CHO)	n.t.	n.t.	n.t.	83
	Q→M	↓5x (CHO)	n.t.	n.t.	n.t.	83
	Q→N	↓2x (CHO)	n.t.	n.t.	n.t.	83
21	K→G	full (EL4)	n.t.	n.t.	n.t.	83
22	T→G	full (EL4)	n.t.	n.t.	n.t.	83
24	Y→G	↓1.6x (EL4)	n.t.	n.t.	n.t.	83
25	L→G	↓2x (EL4)	n.t.	n.t.	n.t.	83
26	R→A	full (CHO)	n.t.	n.t.	n.t.	83
27	N→G	full (EL4)	n.t.	n.t.	n.t.	83
28	N→A	full (CHO)	n.t.	n.t.	n.t.	83
29	Q→G	full (EL4)	n.t.	n.t.	n.t.	83
31	V→G	full (EL4)	n.t.	n.t.	n.t.	83
34	Y→G	↓4-5x (EL4, CHO)	n.t.	↓	no	82, 83

	Y→H	full (EL4, CHO)	n.t.	n.t.	no	82, 83
	Y→K	↓>100x (CHO)	n.t.	n.t.	n.t.	83
	Y→D	↓10x (CHO)	n.t.	n.t.	n.t.	83
	Y→W	full (CHO)	n.t.	n.t.	n.t.	83
	Y→M	full (CHO)	n.t.	n.t.	n.t.	83
36	Q→G	full (EL4)	n.t.	n.t.	n.t.	83
	Q→F	↓>100x (CHO)	n.t.	n.t.	n.t.	83
38	P→G	↓1.4x (EL4)	n.t.	n.t.	n.t.	83
39	N→G	↓1.6x (EL4)	n.t.	n.t.	n.t.	83
40	V→G	full (EL4)	n.t.	n.t.	n.t.	83
41	Q→G	↓1.4x (EL4)	n.t.	n.t.	n.t.	83
43	E→G	↓1.6x (EL4)	n.t.	n.t.	n.t.	83
44	E→G	↓1.6x (EL4)	n.t.	n.t.	n.t.	83
45	K→G	full (EL4)	n.t.	n.t.	n.t.	83
46	I→G	full (EL4)	n.t.	n.t.	n.t.	83
47	D→A	full (CHO)	n.t.	n.t.	n.t.	83
48	V→A	full (CHO)	n.t.	n.t.	n.t.	83
50	P→A	↓1.6x (CHO)	n.t.	n.t.	n.t.	83
52	E→Q	full (EL4)	n.t.	full (LAF, IL-6)	n.t.	Ruggiero unpubl.
	E→A	full (CHO)	n.t.	n.t.	n.t.	83
54	H→G	full (EL4)	n.t.	n.t.	n.t.	83
60	I→A	↓1.4x (CHO)	n.t.	n.t.	n.t.	83
61	H→G	full (EL4)	n.t.	n.t.	n.t.	83
64	K→A	full (CHO)	n.t.	n.t.	n.t.	83
65	M→A	full (CHO)	n.t.	n.t.	n.t.	83
68	S→G	full (EL4)	n.t.	n.t.	n.t.	83
70	V→A	↓1.4x (CHO)	n.t.	n.t.	n.t.	83
71	K→G	full (EL4)	n.t.	n.t.	n.t.	83
72	S→G	full (EL4)	n.t.	n.t.	n.t.	83
74	D→G	↓2-3x (EL4, CHO)	n.t.	n.t.	n.t.	83
75	E→G	full (EL4)	n.t.	n.t.	n.t.	83

76	T→G	full (EL4)	n.t.	n.t.	n.t.	83
79	Q→G	↓2x (EL4)	n.t.	n.t.	n.t.	83
81	E→G	full (EL4)	n.t.	n.t.	n.t.	83
84	N→C	full (CHO)	n.t.	n.t.	n.t.	83
85	I→A	full (CHO)	n.t.	n.t.	n.t.	83
86	T→A	full (CHO)	n.t.	n.t.	n.t.	83
87	D→A	full (CHO)	n.t.	n.t.	n.t.	83
88	L→A	full (CHO)	n.t.	n.t.	n.t.	83
89	S→A	full (CHO)	n.t.	n.t.	n.t.	83
		.				
90	E→A	full (CHO)	n.t.	n.t.	n.t.	83
91	N→R	↑3x (EL4, HaC)	↑2x (1H7)	↑2-5x (LAF, IL-6, Ca) ↑10x (glyc, PMN)	none	Ruggiero unpubl.
	N→G	full (EL4)	n.t.	n.t.	n.t.	83
92	R→D	full (EL4)	n.t.	n.t.	n.t.	83
93	K→A	full (CHO)	n.t.	n.t.	n.t.	83
94	Q→A	full (CHO)	n.t.	n.t.	n.t.	83
95	D→G	full (EL4)	n.t.	n.t.	n.t.	83
96	K→G	full (EL4)	n.t.	n.t.	n.t.	83
97	R→D	full (EL4)	n.t.	n.t.	n.t.	83
101	I→G	full (CHO)	n.t.	n.t.	n.t.	83
102	R→G	↓1.6x (CHO)	n.t.	n.t.	n.t.	83
103	S→G	full (CHO)	n.t.	n.t.	n.t.	83
104	D→G	full (EL4)	n.t.	n.t.	n.t.	83
105	S→A	↑2x (CHO)	n.t.	n.t.	n.t.	83
107	P→G	full (EL4)	n.t.	n.t.	n.t.	83
108	T→G	full (EL4)	n.t.	n.t.	n.t.	83
109	T→A	↑2x (EL4)	↑2x (1H7)	↑2x (LAF, IL-6)	none (LAF, IL-6)	Ruggiero unpubl.
110	S→A	full (CHO)	n.t.	n.t.	n.t.	83
112	E→A	↓1.3x (CHO)	n.t.	n.t.	n.t.	83

113	S→G	full (EL4)	n.t.	n.t.	n.t.	83
117	P→G	↓1.4x (EL4)	n.t.	n.t.	n.t.	83
119	W→G	↓2x (EL4)	n.t.	n.t.	n.t.	83
120	Q→G	full (EL4)	n.t.	n.t.	n.t.	83
125	M→G	full (EL4)	n.t.	n.t.	n.t.	83
126	E→G	full (EL4)	n.t.	n.t.	n.t.	83
128	D→G	↓2x (EL4)	n.t.	n.t.	n.t.	83
129	Q→A	full (CHO)	n.t.	n.t.	n.t.	83
130	P→G	full (EL4)	n.t.	n.t.	n.t.	83
131	V→G	full (EL4)	n.t.	n.t.	n.t.	83
132	S→G	full (EL4)	n.t.	n.t.	n.t.	83
134	T→G	full (EL4)	n.t.	n.t.	n.t.	83
135	N→G	full (EL4)	n.t.	n.t.	n.t.	83
136	M→A	full (CHO)	n.t.	n.t.	n.t.	83
137	P→G	full (EL4)	n.t.	n.t.	n.t.	83
138	D→G	full (EL4)	n.t.	n.t.	n.t.	83
139	D→G	full (EL4)	n.t.	n.t.	n.t.	83
141	V→G	full (EL4)	n.t.	n.t.	n.t.	83
142	M→G	full (EL4)	n.t.	n.t.	n.t.	83
143	V→G	↓1.3x (EL4)	n.t.	n.t.	n.t.	83
144	T→G	full (EL4)	n.t.	n.t.	n.t.	83
145	K→G	full (EL4, CHO)	n.t.	n.t.	no	82, 83
	K→D	full (EL4)	n.t.	yes (D10, GIF, OVCAR)	yes (↓170-1000x E_2, D10) no (GIF, OVCAR)	65, 82, 83, 98, 113
147	Y→G	full (EL4) ↓2-5x (CHO)	n.t.	n.t.	n.t.	83
	Y→K	↓>100x (CHO)	n.t.	n.t.	n.t.	83
	Y→T	↓10-20x (CHO)	n.t.	n.t.	n.t.	83
	Y→H	↓2x (CHO)	n.t.	n.t.	n.t.	83
148	F→A	full (EL4)	n.t.	n.t.	n.t.	83
149	Q→G	↓1.4x (CHO)	n.t.	n.t.	n.t.	83

150	E→A	full (CHO)	n.t.	n.t.	n.t.	83
151	D→A	full (CHO)	n.t.	n.t.	n.t.	83
152	E→A	full (CHO)	n.t.	n.t.	n.t.	83
Multiple modifications						
18/145	V/K→S/D	full (EL4)	n.t.	n.t.	yes (↓170x D10)	113
54/145	H/K→P/D	full (EL4)	n.t.	n.t.	yes (↓85x D10)	113
	H/K→I/D	full (EL4)	n.t.	n.t.	no (↓>4000x D10)	113
91/109	N/T→R/A	↑2-5x (EL4, HaC)	↓10x (1H7)	↑2x (LAF, IL-6) ↑20x (Ca, glyc)	none	Ruggiero unpubl.
108/145	T/K→K/D	↓1.4x (EL4)	n.t.	n.t.	yes (↓570x D10)	113
116/145	C/K→F/D	full (EL4)	n.t.	n.t.	no (↓>4000x D10)	113
122/145	C/K→S/D	full (EL4)	n.t.	n.t.	yes (↓170x D10)	113
	C/K→A/D	full (EL4)	n.t.	n.t.	yes (↓100x D10)	113
145/147	K/Y→D/T	↓>10x (EL4)	n.t.	n.t.	no (↓>4000x D10)	113
	K/Y→D/G	full (EL4)	n.t.	n.t.	no (↓>4000x D10)	113
1-3	des-RPS	full (CHO)	n.t.	n.t.	n.t.	83
1-10	des-RPSGRKSSKM	full (CHO)	n.t.	n.t.	n.t.	83
51-52/145	I-E/K→ins-QGEESN/D (-I*QGGGSND*E-)	full (EL4)	n.t.	n.t.	yes (↓47x D10)	113
53-54/145	P-H/K→ins-QGEESN/D (-P*QGEESN*H-)	full (EL4)	n.t.	n.t.	yes (↓55x D10)	113
51-52/54/145	I-E/H/K→ QGEESN/P/D (-I*QGEESN*E-)	full (EL4)	n.t.	n.t.	yes (↓20x D10)	113

Legend to TABLE V

Position of residues is numbered from the mature IL-1ra sequence, after cleavage of the leader peptide. Amino acids are indicated with the one-letter code.

Binding capacity and biological activities of mutant proteins are indicated in comparison to wild type IL-1ra e.g. "↓2x" stands for a two-fold decrease (i.e. 50%) *vs.* the wild type protein, whereas "↑2x" stands for a doubling of effect *vs.* that of wild type IL-1ra. The inhibitory activity is considered in the fifth column. If agonist activity is acquired, this is shown in the sixth column and compared to the agonist activity of IL-1β.
n.t. = not tested
yes = activity present but not compared to that of the wild type protein
full = 100% of activity of the wild type protein
no or none = no activity detectable.

Binding to IL-$1R_I$ is measured on different cell types, indicated in parentheses:
EL4 = EL4-6.1 murine cells;
CHO = CHO cells transfected with hu r IL-$1R_I$

HaC = human keratinocyte cell line HaCaT.

Binding to IL-$1R_{II}$ is measured on different cell types, indicated in parentheses:
1H7 = 1H7 clone of the human B cell lymphoma RAJI.

Inhibition of IL-1 activities (and IL-1-like agonist activities) are measured in different assays, indicated in parentheses:
EL4 = induction of IL-2 production in EL4 murine cells *in vitro*;
LAF = murine thymocyte proliferation *in vitro*;
IL-6 = induction of IL-6 production *in vitro*;
Ca = modulation of stimulus-induced intracellular Ca^{++} increase *in vitro*;
glyc = induction of hypoglycemia in the mouse *in vivo*;
PMN = induction of neutrophilia in the mouse *in vivo*;
D10 = proliferation of the murine T cell clone D10.G4.1 *in vitro*

GIF = growth inhibition assay on A375 human melanoma cells *in vitro*

OVCAR = inhibition of ovarian carcinoma cells *in vitro*

E_2 = induction of PGE_2 production *in vitro*.

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