INTERLEUKIN-1 FAMILY: FROM GENES TO HUMAN DISEASE

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Interleukin-1 (IL-1) is one of the most potent proinflammatory cytokines involved both in physiological immune responses and in the development of various immunopathological disorders. The members of IL-1 family form a complex system with tight regulatory mechanisms and clarifying their role in disease remains in the centre of research interests. This review article except of summarising recent findings about IL-1 family members focuses on associations of IL-1 gene complex polymorphisms with human diseases.

INTRODUCTION

The immune response is regulated by cytokine network, in which the synergy and antagonism of particular cytokines must be finely tuned to reach the right outcome. Both inadequate and too strong immune reaction can impair the organism and result in inflammatory and autoimmune diseases, respectively. According to their function, cytokines can be divided into groups of proinflammatory or antiinflammatory mediators. One of the key roles in cytokine network is played by the members of the interleukin-1 family. Mediators, belonging to this group, are the proinflammatory agonists IL-1 α , IL-1 β and their naturally occurring inhibitor interleukin-1 receptor antagonist (IL-1Ra). Recently, novel members of IL-1 superfamily, interleukin-18¹, FIL1 δ , FIL1 ϵ , FIL1 ζ , and FIL1 η ² have been described. The members of the IL-1 family show conserved amino acid motifs and share similar tertiary structure. Conserved exon-intron pattern suggests that they evolved from a common ancestor.

IL-1 and IL-1Ra specifically bind to surface receptor molecules or to their soluble forms. There are two receptors binding IL-1. Interaction with the type I receptor (IL-1RI) leads to signal transduction, while type II receptor (IL-1RII) acts as a "decoy" receptor sequestering IL-1 without inducing signalling. An indispensable participant of the IL-1 signalling complex is the IL-1 receptor accessory protein (IL-1RAcP). Novel IL-1 receptor-like genes, the T1/ST2 (IL-1RL1), IL-1 receptor related protein (IL-1Rrp) and IL-1 receptor related protein 2 (IL-1RL2) have been identified during the human genome sequencing project³.

The genes encoding members of IL-1 family are clustered on the long arm of the chromosome 2, except of IL-18 and IL-1RAcP, which are located on the chromosomes 11 and 3, respectively^{3, 4, 5}. IL-1 family genes are polymorphic and their alleles are in linkage disequilibrium⁶.

CHARACTERISTICS OF IL-1 FAMILY MEMBERS

IL-1 α and IL-1 β

Gene. Genes for IL-1 α (IL1A), IL-1 β (IL1B) and IL-1Ra (IL1RN) are located on 2q14-21⁷. They map to a common restriction fragment of approximately 430 kb, where they are distributed in following intervals: IL1A between +0 and +35 kb, IL1B between +70 and +110 kb, IL1RN between +330 and +430 kb⁴. The promoter region of IL-1A does not contain TATA box, a typical motif for inducible genes, which is present in the IL-1B.

Regulation of expression. IL-1 α is constitutively expressed in human keratinocytes, other cells synthesise and release IL-1 α under stimulation^{8, 9}. IL-1 β is produced by wide range of cells, particularly of monocyte-macrophage lineage. There is a large number of microbial or non-microbial inducers of IL-1, such as lipopolysaccharide (LPS), immune complexes, stress factors, neuroactive substances, cytokines^{10, 11}. Constitutive levels of IL-1 β mRNA have been detected in many organs and tissues. However, a dissociation between the transcription and translation is characteristic for IL-1 β ¹². Transcription is induced by various stimulants without translation. The untranslated mRNA is degraded unless an additional signal is provided¹⁰. One possible explanation for this phenomenon is regulation of mRNA half-life through AURE (Adenylate Uridylate Rich Element). IL-1 β mRNA contains an AURE typical for mRNAs, which require tight control of protein synthesis¹¹.

Synthesis and processing. Both IL-1 α and IL-1 β are synthesised as 31-kDa precursors without conventional signal peptide, characteristic for secreted proteins¹⁰. Mature 17-kDa forms of IL-1 α and IL-1 β have a trefoil-like tri-dimensional structure composed of 12 to 14 folds of β -pleated sheet. The protein precursors are translated on polysomes in connection with cytoskeletal microtubules, remain in cytosol and do not accumulate in any organelle. Either proIL-1 α or proIL-1 β are

myristoylated on lysine residues during the process of posttranscriptional modification¹³. Myristoylated proIL-1 α is translocated to the cell surface or can be cleaved to a mature form by membrane-associated calcium-dependent cysteine proteases called calpains¹⁴. After cleavage IL-1 α is released to extracellular space. IL-1 α is fully active both as precursor or mature protein, whereas IL-1 β is active only in its mature form. In keratinocytes and partially in tissue macrophages, the predominant form of IL-1 β after stimulation is intracellular proIL-1 β ¹⁵. The active mature 17-kDa IL-1 β arises from cleavage of proIL-1 β at aspartic acid at position 116 by interleukin-1 β converting enzyme (ICE, recently called caspase-1)¹⁶. In mononuclear phagocytes, the ICE cleavage of proIL-1 β also at Asp²⁸ was demonstrated. Processing of proIL-1 β is not indispensable for IL-1 β export into extracellular compartment. IL-1 β is secreted from intact LPS-stimulated monocytes as 3-, 17-, 28- and 31-kDa forms¹⁵. The released 31-kDa form is different from cytosolic 31-kDa proIL-1 β , what could be explained by conformational changes during the process of secretion. Apart from ICE, proIL-1 β can be cleaved by other proteolytic enzymes, such as trypsin, chymotrypsin, bacterial enzymes, leukocyte elastase, granzyme A, and matrix metalloproteinases (MMPs)¹⁷. MMPs including stromelysin-1, gelatinases A and B, are induced by IL-1 β at sites of acute and chronic inflammation, where they are able both to activate and catabolise IL-1 β .

Function. The biologic effects of IL-1 have been described in detail in previous reviews¹⁰. IL-1 α acts as an intracellular autocrine messenger, which regulates the normal cell differentiation, especially in epithelial and ectodermal cells. ProIL-1 α NH₂ terminal region contains a nuclear localisation sequence and has been demonstrated to bind in cell nuclei¹⁸. Elevated levels of IL-1 α were proposed to play role in senescence and growth inhibition of fibroblasts and endothelial cells. The critical role of IL-1 β , but not of IL-1 α , in febrile and neuro-immune-endocrine responses was described in IL-1 α -/-, IL1 β -/- knockout (KO) mice¹⁹. IL-1 β is crucial for the induction of fever and acute-phase response during local tissue damage, whereas in systemic inflammation it only contributes to inflammatory reaction^{20,21}. Similar to IL-1 β -/- mice, IL-1RI-deficient animals have a reduced acute phase response to local injury. Data obtained in studies with IL-1RI-deficient mice suggest that both IL-1 α and IL-1 β are important in response to tissue damage and infection, but are not required for normal development and homeostasis²². In the response to inflammatory and infectious challenges IL-1 modulates the process of extravasation and localisation of leukocytes at inflammatory sites, which involves leukocyte adhesion and passage through endothelial linings¹⁰. IL-1 participates in recruiting and activating neutrophils and macrophages, producing vascular dilatation with alteration of endothelial integrity. It also induces production of acute phase reactants, wide range of cytokines, adhesive molecules, prostanoids, free-radical generating enzymes etc. Further, IL-1 enhances pro-

liferation of fibroblasts, smooth muscle cells or mesangial cells¹⁰.

IL-1Ra

IL-1 receptor antagonist has currently three known isoforms: glycosylated soluble IL-1Ra (sIL-1Ra) and two intracellular non-glycosylated forms (icIL-1Ra I and icIL-1Ra II).

Gene. sIL-1Ra and icIL-1Ra I are generated from the same gene by use of alternative first exon²³. The expression of sIL-1Ra and icIL-1Ra I is regulated by two distinct promoters located upstream to each alternative first exon with different transcriptional regulatory elements. The regulation of icIL-1Ra expression is realised in cell type-specific manner²⁴.

Structure. IL-1Ra has a β -trefoil crystallographic structure, which is similar to those of IL-1²⁵. The characteristics of particular isoforms are listed in Table 1. sIL-1Ra is translated as a 17-kDa protein with 177 amino acids containing a 25-amino acid leader sequence¹⁰. After processing, which includes cleavage of the leader sequence and glycosylation, it is secreted by classical endoplasmatic reticulum – Golgi – secretory vesicles pathway into the extracellular environment. Intracellular forms of IL-1Ra are not glycosylated, do not possess leader sequence and remain in intracellular compartment. icIL-1Ra II is derived by alternative translation initiation from either sIL-1Ra mRNA and icIL-1Ra I mRNA²⁶. Two different icIL-1Ra II have been described. mRNA coding for a larger 25-kDa form of icIL-1Ra was found in human polymorphonuclear cells, which differed from icIL-1Ra I in additional 21 amino acid sequence present within the NH₂-terminal portion²⁷. This additional stretch was encoded by an extra exon located 2kb downstream between the first and second exon of icIL-1Ra I. Even if this mRNA is expressed in several cell types, the corresponding 25-kDa protein has not been found yet²⁸.

Table 1. Characteristics of IL-1Ra isoforms.

isoform	molecular weight	number of amino acids	Producing cell types
sIL-1Ra	22-25kDa	152	monocytes, macrophages, neutrophils, fibroblasts, hepatocytes, microglia
icIL-1Ra I	18kDa	159	keratinocytes, epithelial cells, monocytes, macrophages
icIL-1Ra II	16kDa	143	fibroblasts, keratinocytes, epithelial cells, neutrophils, myelomonocytes, hepatoma HepG2 cells

Abbreviations: sIL-1Ra, soluble IL-1 receptor antagonist; icIL-1Ra I, intracellular IL-1 receptor antagonist type I; icIL-1Ra II, intracellular IL-1 receptor antagonist type II.

Regulation of production and function. IL-1Ra production is induced by variety of mediators including cytokines, viral products, and acute phase proteins. The most potent inducers of IL-1Ra *in vitro* are LPS, IL-4, adherent IgG and GM-CSF²⁸. Differential production of distinct IL-1Ra isoforms by LPS-stimulated peripheral mononuclear blood cells (PMBC) and neutrophils

was demonstrated²⁹. sIL-1Ra is an inducible gene in most cells, icIL-1Ra is expressed constitutively in keratinocytes and intestinal epithelial cells¹⁰. The early transcription and translation of sIL-1Ra both in polymorphonuclear leukocytes and in PBMC may regulate the biological activity of IL-1 in extracellular space. The transcription and translation of icIL-1Ra I in PBMC is delayed, and possibly it has an intracellular regulatory function. In transduced human ovarian carcinoma cells icIL-1Ra I does not inhibit initial IL-1 β responses, but attenuates IL-1 β -inducible early gene expression by alteration of mRNA stability³⁰. The existence of intracellular regulatory loops modulating the activities of intracellular IL-1 family members has been suggested³¹. In cultured dermal fibroblasts from patients with systemic sclerosis elevated intracellular proIL-1 α up-regulates the levels of icIL-1Ra. icIL-1Ra II is produced by neutrophils and PBMC as an inducible cytoplasmic protein, which follows the kinetics of sIL-1Ra²⁹. The physiological inhibition of IL-1 by IL-1Ra appears to protect the organism from exaggerated reaction to infection, but brings along the risk of impairing the host's ability to eliminate the infection. The IL-1Ra KO mice are more susceptible to lethal endotoxemia but are less susceptible to infection with intracellular agent *Listeria monocytogenes*³². The absence of IL-1Ra in KO mice resulted also in decreased body mass compared with wild-type controls suggesting that IL-1Ra is not required for normal development, but it is necessary for maintaining normal body mass by mechanism antagonising the IL-1-mediated appetite suppression and catabolism³².

IL-1 receptors

Six members of the interleukin-1 receptor family are currently known³. The family contains: type I IL-1 receptor (IL-1RI), type II IL-1 receptor (IL-1RII), IL-1 receptor accessory protein (IL-1RAcP), T1/ST2 (IL-1RL1), IL-1 receptor related protein (IL-1Rrp) and IL-1 receptor related protein 2 (IL-1RL2). The extracellular portion of these receptors is composed of three IgG-like domains which share significant homology to each other^{3,10}. Five of six IL-1 receptor-like genes map to 2q12, while the IL-1RAcP to 3q28.

IL-1 receptor type I. IL-1RI is a transmembrane monomeric 80-kDa glycoprotein, constitutively expressed on T lymphocytes, fibroblasts, chondrocytes, endothelial cells, smooth muscle cells and other cell types²⁵. Signal transduction via IL-1RI requires recruitment of IL-1RAcP, which is an essential signal transducing component in functional IL-1R complex³³.

IL-1 receptor accessory protein. IL-1RAcP is a 570-amino acid protein, which shares limited homology to both IL-1 receptors. The conformational changes induced in ligand-receptor complex by tight binding of the IL-1 agonists to the second binding site in the domain 3 of the receptor (which is not present in IL-1Ra), allow a secondary interaction of this complex with IL-1RAcP²⁸. IL-1RAcP increases the affinity of the IL-1RI

for all IL-1 agonists, but differentially affects binding of IL-1 α and IL-1 β ³³. IL-1Ra binds to extracellular portion of IL-1RI without triggering the association of IL-1RAcP.

IL-1 receptor type II. IL-1RII is a 68-kDa glycoprotein with shortened intra-cytoplasmic domain in comparison with IL-1RI³⁴. It is expressed on the same cells as the IL-1RI with the exception of endothelial cells²⁵. IL-1RII is unable of signal transducing and competes with IL-1RI for IL-1. IL-1RII does not act only as a scavenger of IL-1 but also of IL-1RAcP. IL-1RII alters IL-1 activity by subtracting IL-1RAcP from IL-1RI signalling complex³⁵. Thus the IL-1RI / IL-1RII ratio on the cell membrane is an important factor regulating IL-1 cell responsiveness. Expression of IL-1RII is modified by diverse external signals. Particularly anti-inflammatory agents (IL-4, IL-13, dexamethason) can up-regulate IL-1RII surface expression in human macrophages and neutrophils³⁶.

Soluble forms of receptors. The soluble forms of IL-1RI and II are derived by proteolytic cleavage of extracellular domains of membrane receptors. They can be detected in the serum or urine of healthy subjects, but also in different inflammatory body fluids³⁷. The levels of sIL-1RII are higher than sIL-1RI both in normal and pathologic situations. The affinity of soluble receptors for IL-1 and IL-1Ra is different from membrane-bound receptors. For sIL-1RI, the rank is IL-1Ra > IL-1 α > IL-1 β , for sIL-1RII IL-1 β > IL-1 α > IL-1Ra^{10,25}.

Signal transduction. The binding of IL-1 to IL-1RI allows the association of IL-1RAcP, thus forming a high-affinity complex for IL-1³⁵. The ligand-induced association of two transmembrane molecules results in the interaction of their cytoplasmic domains necessary for the recruitment of the IL-1 receptor associated protein kinases (IRAK, IRAK-2, IRAK-M)^{38, 39, 40}. IRAK then dissociate from the IL-1R complex and associate with TNF receptor-associated factor-6 (TRAF-6). The members of the IRAK family are indispensable elements for LPS signal transduction⁴⁰. Events including multiple and sequential phosphorylations and dephosphorylations of distinct kinases (e.g. MAP kinases) result in activation of proteins participating in translation and translocation of transcriptional factors (particularly NF κ B and AP-1) to the nucleus. A member of Rho family of GTPases - RhoA appears to constitutively interact with the cytoplasmic domain of IL-1RI as a component of the IL-1 signalling complex. The activation of RhoA leads to IL-1-stimulated Rho-dependent cytoskeleton reorganisation, which is necessary for persistent cell activation in chronic inflammatory disease⁴¹.

IL-18

IL-18, previously called IFN γ -inducing factor, has been classified to IL-1 family on the basis of its structural homology. Contrarily to other IL-1 family members, the gene of IL-18 maps to 11q22.2-q22.3⁵. IL-18 is constitutively expressed in many haematopoietic cell lines of myelomonocytic origin, murine splenocytes, osteo-

blasts, mouse keratinocytes, rat adrenal cortex and pituitary gland⁴². It is synthesised as an inactive precursor molecule lacking signal peptide, then processed by ICE⁴². IL-18 is active in complex with its receptor. IL-18 receptor (IL-18R) consists of binding chain IL-18R α , formerly identified as the IL-1 receptor-related protein (IL-1Rrp), and a signalling chain IL-18R β . The signal transduction cascade is similar to that of IL-1. The IL-18R complex recruits IRAK and TRAF-6⁴³. These events lead to activation of NF- κ B. IL-18 induces IFN- γ and its biological effects are similar to IL-12 in mediating T_H1 response and activation of natural killer cells. It also induces gene expression and synthesis of TNF, IL-1, Fas ligand and of some chemokines⁴⁴.

FIL1 δ , FIL1 ϵ , FIL1 ζ , FIL1 η

These novel members of IL-1 superfamily were recognised by searching the public EST databases relying on the sequence similarities². Sequence alignment revealed approximately a 20–35% sequence identity between the IL-1 superfamily members. A most common shared amino acid motif is F(X_{10–12})FXS(AVS)XX(PE)XX(FY)(LI)(CAS) (TC) with one-letter amino acid code, where X is any amino acid. According to the similarity, the members of the superfamily can be divided into three subgroups. The first includes IL-1 α , IL-1 β , IL-18, the second IL-1Ra, FIL δ and third FIL ζ , FIL ϵ , FIL η ². The tertiary structure of the novel members is also a 12-stranded β -trefoil, which is well conserved in IL-1. The major difference between FIL δ , FIL ϵ , FIL1 ζ is in loops connecting the β strands. The novel family members are expressed in different haematopoietic and non-haematopoietic cell types. All of them are expressed in activated monocytes and B cells, FIL1 ϵ also in T cells. Their biologically activity is unknown.

RELATIONSHIP BETWEEN IL-1 FAMILY MEMBERS

The mechanism of action and relationship between IL-1 and their counterparts has several levels. In the synthesis pathway, the production of the agonists and antagonists is regulated at the level of pre-transcriptional processes, transcription, post-transcriptional mRNA splicing, mRNA stability, translation and processing. IL-1 β is an auto-regulating protein, inducing its own expression in human PBMC and endothelial cells *in vitro*⁴⁵. This auto-induction can be inhibited by IL-1Ra, thus suggesting IL-1Ra is a negative regulator of positive feedback loop of IL-1 production⁴⁶. However, in IL-1Ra knockout and overproducing mice it was found, that IL-1Ra up-regulates the IL-1 serum levels during endotoxemia³². It is plausible that IL-1Ra *in vivo* acts both as a positive and negative regulator of IL-1 expression.

Fig. 1 Model of interaction between IL-1, IL-1Ra and IL-1 receptors. Arrows represent signal transduction. Abbreviations: IL-1 α , interleukin-1 α ; IL-1 β , interleukin-1 β ; IL-1Ra, IL-1 receptor antagonist; IL-1RI, IL-1 receptor type I; IL-1RII, IL-1 receptor type II; sIL-1RI, soluble IL-1 receptor type I; sIL-1RII, IL-1 receptor type II.

Beyond these events the bioavailability and bioactivity of IL-1 is regulated by specific inhibitors, cell-bound or soluble IL-1 receptors (Figure 1). IL-1RI has approximately equal affinity for IL-1 α , IL-1 β and IL-1Ra¹⁰. Despite near equal affinities, IL-1Ra needs large molar excess (10- to 100-fold) to inhibit the IL-1 agonistic action⁴⁷. IL-1Ra associates with IL-1RI slower than IL-1, and IL-1 rather than IL-1Ra occupies the empty receptor. IL-1RII has the affinity for IL-1 β 100-fold stronger than for IL-1Ra⁴⁸ and its preference range is IL-1 β > IL-1 α > IL-1Ra¹¹. Thus the IL-1RII blocks IL-1 β binding to signal transducing IL-1RI. As for the soluble forms, sIL-1RI binds preferentially IL-1Ra to IL-1 α and IL-1 β . Under normal conditions it does not alter the availability of IL-1 β to membrane-bound IL-1RI, but neutralises the antiinflammatory action of IL-1Ra. On the other hand sIL-1RII with great affinity to IL-1 β sequesters IL-1 β and disrupts its activity. The great excess of sIL-1RII to sIL-1RI in extracellular fluid supports the idea, that sIL-1RII, rather than sIL-1RI, is the major regulator of IL-1 β activity¹¹.

IL-1 FAMILY IN DISEASE

The pivotal role of IL-1 in various disorders has been demonstrated in numerous studies with human diseases or experimental animal models of disease. They have been summarised in previous reviews^{10, 28}. Beneficial effects of administration of recombinant IL-1Ra in animal models have enabled using IL-1Ra treatment in human clinical trials.

Autoimmune diseases

The involvement of IL-1 in the pathogenesis of *rheumatoid arthritis* (RA) is now well accepted. IL-1 appears to be important in inflammation-induced erosive cartilage damage and bone resorption in animal polyarthritis models, but it is less involved in the early joint swelling and periarticular inflammation^{49, 50}. In rat type II collagen-induced arthritis with lesions analogous to those in human RA, IL-1 is a major mediator participating in all aspects of disease progression⁵⁰. Clinical studies suggested a correlation between levels of IL-1 β and parameters of joint damage⁵¹. Thus inhibiting the IL-1 activity may be beneficial in treatment of RA. The important regulatory role of IL-1Ra in disease has been shown in IL-1Ra deficient mice, where a chronic inflammatory arthropathy with autoimmunity has developed, resembling human RA⁵². Blocking IL-1 with anti-IL-1 α + β treatment during murine type II collagen-induced arthritis protects the cartilage and bone structures⁵³. The administration of IL-1Ra to RA patients in clinical trials have resulted in decrease of inflammatory disease activity and may inhibit the progression of joint erosion⁵⁴.

In *systemic lupus erythematosus* (SLE) high serum concentration of IL-1Ra has been measured as a pathophysiologic feature of disease activity⁵⁵. However, there are contradictory findings about the expression of IL-1Ra by PBMC in response to adherent IgG in active SLE. Suzuki et al. (1995) report enhanced expression of IL-1Ra mRNA in freshly isolated monocytes, while recently a deficiency in Fc γ R-mediated production of IL-1Ra in monocytes from patients with active SLE compared with those with inactive disease was found⁵⁶. This deficiency may contribute to immune-complex-mediated tissue damage in SLE.

The dermal fibroblasts from patients with *systemic sclerosis* (SSc) exhibit an aberrant constitutive expression of IL-1 α mRNA and contain intracellular proIL-1 α ⁵⁷. SSc fibroblasts are characteristic by increased production of IL-1 α , IL-6 and PDGF-A, the key mediators of collagen synthesis, which mediate an excessive extracellular matrix synthesis⁹.

IL-1 β plays a crucial role also in "destructive" organ-specific autoimmunity in *Hashimoto's thyroiditis* by induction of efficient autoantigen presentation via costimulatory molecules and Fas/FasL-mediated apoptosis of thyroid follicular cells⁵⁸.

Gastrointestinal tract

In peripheral blood or biopsy specimen from patients with *inflammatory bowel diseases* (IBD) increased amounts of IL-1 and IL-1Ra have been measured^{59, 60, 61}. The increased capacity of cultured colonic lamina propria mononuclear cells to produce IL-1 β has been shown to predict acute relapses in Crohn's disease⁶². Both in ulcerative colitis and Crohn's disease a decreased ratio of mucosal IL-1Ra / IL-1 β at protein and mRNA levels has been described, correlating with disease severity⁶³. Even if the expression of IL-1Ra in intestinal mucosa is

enhanced, its production is relatively low to that of IL-1 β and this imbalance between IL-1 β and IL-1Ra production appears to be decisive in IBD. The importance of IL-1Ra has been demonstrated in experimentally induced colitis in rabbits, where administration of anti-IL-1Ra antibodies increased mortality and prolonged intestinal inflammatory responses⁶⁴.

Higher serum levels of IL-1 β and IL-1Ra were found also in *chronic liver diseases*. In HCV-related chronic autoimmune hepatitis an increased IL-1 β / IL-1Ra mRNA ratio at tissue level appears to contribute to the development of more aggressive disease forms with higher grade of fibrosis⁶⁵.

Using several animal models of *acute pancreatitis* (AP), high concentration of IL-1 in pancreatic parenchyma, but also in other organs were measured. It was found that IL-1 does not trigger acute pancreatitis, but plays important role in disease progression⁶⁶.

Lung

In established *acute respiratory distress syndrome* (ARDS), elevated levels of both IL-1 β and IL-1Ra were found in alveolar air space and plasma^{67, 68, 69}. The systemic level of IL-1 β seems to reflect the severity of the lung injury rather than the diagnosis in different lung diseases⁷⁰. The levels of IL-1 β in BAL fluid from patients with ARDS did not differ between survivors and non-survivors, but low concentrations of anti-inflammatory cytokines IL-1Ra and IL-10 were associated with worse prognosis⁶⁸. These observations support the hypothesis that inability to intensify the local anti-inflammatory activity early in the development of ARDS contributes to more severe lung injury and has prognostic significance. In animal ischemia/reperfusion acute lung injury model IL-1 mRNA in lung tissue is up-regulated after transient lung ischemia in a time-dependent manner⁷¹. In loci of hypoxic vascular injury IL-1 participates in attraction of leukocytes. IL-1 β in concert with TNF α is considered to play a central role also in pathogenesis of acute pancreatitis associated ARDS^{72, 73}. Up-regulation of IL-1 and TNF α production within the lung parenchyma is present after intravenous infusion of sterile pancreatic ascites from AP animals free of IL-1 β , IL-6, IL-8, TNF- α , INF- γ and LPS, thus suggesting that these mediators are not likely to be directly responsible for induction of ARDS during AP⁷³. However, IL-1 and TNF- α genes are induced in a delayed fashion after the onset of acute pancreatitis and are required for development of sepsis associated ARDS.

Furthermore, high concentration of IL-1 β in bronchoalveolar lavage (BAL) fluid have been found in *allergic responses* and *bronchial asthma*⁷⁴. The cytokine release in the airways results from modulatory interaction between epithelial cells, alveolar macrophages and infiltrating monocytes.

In BAL fluid of patients with *cryptogenic fibrosing alveolitis* (CFA) an increased level of IL-1Ra has been reported accompanied by proportional elevation of IL-1 β ⁷⁵. A significant increase of IL-1Ra was also present in

tissue homogenates, but without concomitant elevation of IL-1 β . Hyperplastic type II pneumocytes, pulmonary fibroblasts and macrophages have been identified as the primary source of IL-1Ra. These findings demonstrate production of IL-1Ra by non-immune cells and suggest a possible role of IL-1Ra in disease progression through attenuation of IL-1-dependent normal tissue remodeling during resolution and tissue repair. In contrast, other studies describe decreased amounts of IL-1Ra in culture supernatants from CFA patients⁷⁶. There are no differences in number or affinity of type I IL-1 signal transducing receptor on lung fibroblasts between CFA patients and controls⁷⁷.

In *sarcoidosis*, alveolar macrophages *in vitro* spontaneously release greater amounts of IL-1 β in active disease in comparison with cells from patients with inactive disease and from healthy controls⁷⁸.

Sepsis

Serum levels of IL-1 β and IL-1Ra are significantly elevated in severe sepsis and the higher levels of IL-1Ra are associated with an early hemodynamic deterioration⁷⁹. Both the pro- and anti-inflammatory response is augmented in sepsis with imbalance of counteracting mediator systems. Studies with IL-1RI KO mice showed that IL-1 significantly contributes to sepsis-induced endothelial damage⁸⁰. IL-1 β , derived from Kupffer cells, appears to be directly or indirectly responsible also for hepatocellular dysfunction occurring early after the onset of sepsis⁸¹.

Central nervous system

IL-1 β has been implicated as a mediator of *hypoxic-ischemic (HI) brain injury*, both in experimental models and in human⁸². Brain IL-1 β activity and mRNA levels increase acutely after unilateral cerebral HI in neonatal rats⁸³. The absence of IL-1 β processing by ICE in neonatal ICE-deficient mice have increased the resistance to HI brain injury. These findings suggest that ICE is crucial in the evolution of ischemic brain damage leading to neurologic deficits in perinatal period⁸².

The importance of ICE has been demonstrated also in early phase of immune-mediated demyelination of the CNS, where reduction of experimental *autoimmune encephalomyelitis* incidence and severity in ICE -/- mice was observed⁸⁴.

GENE POLYMORPHISMS OF THE IL-1 COMPLEX

Influence of gene polymorphisms on IL-1 protein expression

Cytokines and cytokine receptors are generally highly conserved in their coding regions. Polymorphisms within the exons directly affect the protein structure and possibly result in alteration of protein function. Mutations not affecting the sequence of amino acids in polypeptide chains may influence protein production on diffe-

rent transcriptional or posttranscriptional levels⁸⁵. They can alter the structure of genes' regulatory elements with binding sites for transcriptional factors, transcription rate, mRNA splicing or mRNA stability. For example, in case of VNTR polymorphism of IL-1 α gene in intron 6, each of the repeat sequences contains binding sites for transcription factor SP1, an imperfect copy of a viral enhancer element and an inverse and complementary sequence of a glucocorticoid-responsive element⁸⁶. Table 2 contains the known gene polymorphisms of IL-1 complex.

Table 2. The gene polymorphisms of IL-1 family members^{85, 86, 97, 98, 99, 100, 101}.

Gene	Single base substitution			VNTR polymorphism	
	position	exchange	RFLP	position	repeat sequence
IL-1 α	-889 +4345	C/T T/G		intron 6	46 bp (5,8,9,12,15,18 repeats) dinucleotide repeats (TTA) repeats
IL-1 β	-511 +3953 -31 nt5810	C/T C/T T/C G/A	AvaI TaqI AluI BsoFI		
IL-1Ra	+2016 nt8006 nt8061 nt9589 nt11100	T/C T/C C/T A/T T/C	MspI MwoI SspI MspAII	intron 2	86 bp (2,3,4,5,6 repeats)
IL-1RI	exon 1B exon 1C exon 1C intron 1B	C/T A/T C/A A/G	PstI (A) PstI (B) BsrBI MspI		

Abbreviations: RFLP, Restriction Length Fragment Polymorphism; VNTR, Variable Number of Tandem Repeats; IL-1 α , interleukin-1 α ; IL-1 β , interleukin-1 β ; IL-1Ra, IL-1 receptor antagonist; IL-1RI, IL-1 receptor type I; C, cytosine; T, thymine; A, adenine; G, guanine.

In vitro and *in vivo* secretion of IL-1 β and IL-1Ra exhibits interindividual variability associated with gene polymorphisms. The presence of allele 2 of IL-1 β single base substitution polymorphism at position +3953 may increase *in vitro* LPS-stimulated IL-1 β production by PMBC, where an allele-dosage effect was observed⁸⁷. Allele 2 of IL-1Ra VNTR polymorphism (IL-1RN*2) is suggested to be the major up-regulator of IL-1 β ⁸⁸ and IL-1Ra *in vitro* levels in stimulated mononuclear cell cultures⁸⁹. However, contradictory observations have been described. The carriage of IL-1RN*2 has been associated with decreased production of total IL-1Ra by PBMC from ethnically different ulcerative colitis patients and healthy controls⁹⁰. IL-1 β +3953 and -511 single base substitution polymorphisms also participate in the regulation of IL-1Ra *in vivo* production and they show a cooperative effect with IL-1RN*2⁹¹. IL-1RN*2 phenotype increases IL-1Ra levels *in vivo* in healthy population only in the presence of allele 2 of IL-1 β -511. Allele 2 of IL-1 β -511 and IL-1RN*2 are considered to represent a "high secretor" phenotype leading to increased proinflammatory activity in autoimmune and infectious diseases⁹². More than the absolute amount of IL-1 β or IL-1Ra, the IL-1 β / IL-1Ra ratio is decisive in inflammatory response.

Table 3. Associations of IL-1 complex gene polymorphisms with disease

Disease	Gene polymorphism	Association	Reference
alopecia areata	IL-1RN	+	102
autoimmune hepatitis	IL-1β +3953	-	103
	IL-1RN	-	103
autoimmune hypothyroidism	IL-1RN	-	104
CMV seronegativity	IL-1RN, TNFα -308 #	+	105
Crohn's disease	IL-1β -511	-	94
	IL-1RN	-	106
	IL-1RN	-	94
cryptogenic fibrosing alveolitis	IL-1RN	+	107
diabetic nephropathy in IDDM	IL-1α -889	-	108
	IL-1β +3953	+	108
	IL-1β +3953	-	109
	IL-1RI (PstI)	-	109
	IL-1RN	+	110
	IL-1RN	-	108
	IL-1RN	-	109
early postmenopausal bone loss	IL-1RN	+	111
early-onset pauciarticular	IL-1α -889	+	112
juvenile rheumatoid arthritis	IL-1α -889	-	113
psoriasis	IL-1RN	+	114
EBV seronegativity	IL-1β -511	+	115
Graves' disease	IL-1RN	-	104
	IL-1RN	-	116
Henoch-Schönlein nephritis	IL-1RN	+	117
IDDM	IL-1RI (PstI)	+	118
lichen sclerosus	IL-1RN	+	119
multiple sclerosis	IL-1RN	+	120
	IL-1RN	+	121
	IL-1β +3953, IL-1RN #	+	122
myasthenia gravis	IL-1β +3953	+	123
	IL-1RN	-	123
periodontitis	IL-1β +3953	+	124
	IL-1α -889, IL-1β +3953 #	+	125
primary Sjögren's syndrome	IL-1RN	+	126
	IL-1RN	-	127
rheumatoid arthritis	IL-1α -889	+	128
secondary acute myeloid leukaemia	IL-1RN	-	129
severe sepsis	IL-1β +3953	-	130
	IL-1RN	+	130
schizophrenia	IL-1β +3953	-	131
	IL-1β -511	-	132
	IL-1α -889, IL-1β -511, IL-1RN #	+	133
systemic lupus erythematosus	IL-1RN	+	134
	IL-1RN	-	104
tuberculosis	IL-1RN	+	135
ulcerative colitis	IL-1β +3953	-	136
	IL-1β +3953	-	94
	IL-1β -511	+	137
	IL-1β -511	-	94
	IL-1RN	+	138
	IL-1RN	+	94
	IL-1RN	+	90
	IL-1RN	-	95
	IL-1RN	-	106

haplotype, * disease severity

Abbreviations: IL-1α -889, single base substitution polymorphism at position -889 in the IL-1α gene; IL-1β -511, single base substitution polymorphism at position -511 in the IL-1β gene; IL-1β +3953, single base substitution polymorphism at position +3953 in the IL-1β gene; IL-1RN, variable number tandem repeat polymorphism in intron 2 of the IL-1Ra gene; TNFα -308, single base substitution polymorphism at position -308 in the tumour necrosis factor-α gene; IL-1RI (PstI), PstI polymorphism in the IL-1 receptor type I gene.

Association of IL-1 complex gene polymorphisms with human diseases

The investigated associations between gene polymorphisms of IL-1 complex and human diseases are listed in the Table 3. The polymorphisms of IL-1 family genes are implicated especially in the development of autoimmune, chronic inflammatory diseases and malignancies. In many cases the frequency of an allele or a haplotype does not differ between healthy controls and patients, but there is a significant difference in distinct

forms of disease according to the severity and/or progression. Gene polymorphisms may be markers of susceptibility, severity or may predict the clinical outcome. Particular ethnic differences in distribution of distinct alleles of gene polymorphisms have been observed. In ulcerative colitis (UC), investigated in Caucasoid populations, a weak (patients from U.K.) or no significant (patients from France, Germany, Netherlands) association was found between IL-1RN*2 and susceptibility to UC^{93, 94, 95, 96}. In contrast, a strong association has been described in Hispanic-Caucasian and Jewish-Caucasian ethnic groups⁹⁰. It is also suggested, that IL-1RN*2 is not directly responsible for the disease predisposition, but is in linkage disequilibrium with another DNA sequence variation within or close to the IL-1RN gene, which is more directly responsible for susceptibility of an individual to UC.

CONCLUSION

Interleukin-1 family is a rather complex self-regulating system, which affects almost all cell types in organism. Its members have been shown to be important either in health and in the development of various disorders. Elucidating the mechanism of action of this cytokine family may help to understand the aetiology and pathophysiology of human diseases and subsequently establish novel strategies for primary prevention and/or therapeutic intervention. Measuring IL-1 production in disease states may serve as a marker of the clinical course of disease or therapeutic efficacy. Since IL-1 family members have been proposed to be the candidate genes in many polygenic disorders, their gene polymorphisms are important in obtaining an insight into the genetic background of human immunopathologies.

ACKNOWLEDGEMENTS

The author thanks to Dr. M. Petřek for his complex guidance throughout preparation of this manuscript and also to Dr. J. Drábek for his critical review of this paper. The work was supported by Grant Agency of Czech Republic (No 310/99/1676) and Medical Faculty of Palacký University (No 14501103).

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