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Minireview

Interleukin-8, a chemotactic and inflammatory cytokine

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Interleukin-8 (IL-8) belongs to a family of small, structurally related cytokines similar to platelet factor 4. It is produced by phagocytes and mesenchymal cells exposed to inflammatory stimuli (e.g., interleukin-1 or tumor necrosis factor) and activates neutrophils inducing chemotaxis, exocytosis and the respiratory burst. In vivo, IL-8 elicits a massive neutrophil accumulation at the site of injection. Five neutrophil-activating cytokines similar to IL-8 in structure and function have been identified recently. IL-8 and the related cytokines are produced in several tissues upon infection, inflammation, ischemia, trauma etc., and are thought to be the main cause of local neutrophil accumulation.

Interleukin 8; Receptor; Biological function; Neutrophil activation; Pathophysiology

1. INTRODUCTION

The accumulation of neutrophil leukocytes in a tissue is the hallmark of inflammation, a defence reaction to infection or other injury, with participation of blood, blood vessel and tissue elements. The neutrophils are recruited from the blood by chemotactic stimuli that direct their migration through the vessel wall to the affected site.

Interleukin-8 (IL-8) was identified in 1987 as a novel type of neutrophil-activating cytokine [1]. Today we know its sequence and 3D structure, the sequence and binding properties of its receptors, and the profile of biological activity. Several additional cytokines related to IL-8, but arising from different genes have also been characterized. IL-8 and related cytokines are released by phagocytes and a wide variety of tissue cells upon exposure to inflammatory stimuli. They are the main tissue-derived chemoattractants for neutrophils.

2. PROPERTIES

2.1. *Structure*

IL-8 is generated as a precursor of 99 amino acids and is secreted after cleavage of a signal sequence of 20 residues. N-terminal extracellular processing of the mature form yields several biologically active variants (Fig. 1). The predominant variant consists of 72 amino acids and has a molecular weight of 8,383. It is a basic protein (pI 8.3) and contains four cysteines that form two disulfide bridges. It is resistant to plasma peptidases, heat, pH extremes and other denaturing treatments, but is rapidly inactivated when the disulfide bonds are reduced [1]. Nuclear magnetic resonance spectroscopy and X-ray crystallography studies indicate that IL-8 forms a dimer [2]. The monomer has a short, conformationally flexible N-terminal domain that is anchored by the two disulfide bonds to a core structure consisting of three antiparallel β-strands followed by a terminal α-helix (Fig. 2).

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2.2. *Actions on neutrophil leukocytes*

IL-8 was originally recognized as a neutrophil-activating protein on the basis of two in-vitro effects, chemotaxis and the release of granule enzymes [1]. These effects suggested that IL-8 was a novel type of chemoattractant, and for this reason it was compared extensively with well established chemotactic agonists, such as C5a, fMet-Leu-Phe, platelet-activating factor (PAF) and leukotriene B_4 (LTB_4). These stimuli induce three main responses in neutrophils: (i) shape change and directional migration, (ii) exocytosis of storage proteins, and (iii) the respiratory burst.

The shape change reflects the activation of the contractile system and enables the neutrophils to adhere to endothelial cells and to migrate. In neutrophil suspensions, the shape change can be monitored in a laser nephelometer as a transient rise in light transmission, which reflects the decrease in cell body volume consequent to the formation of large and thin lamellipodia [3]. The shape-dependent transmission increase in response to IL-8 is virtually instantaneous and is similar in kinetics and extent to that observed with C5a or

fMet-Leu-Phe. When the response of the cells is synchronized by prestimulation, the transmission changes induced by chemoattractants including IL-8 show regular oscillations that correlate with the assembly and disassembly of filamentous actin, and are thought to reflect the protrusion and retraction of lamellipodia [3].

Exocytosis leads to the release of soluble storage proteins and the remodeling of the neutrophil plasma membrane by fusion with the storage organelles. IL-8 triggers the exocytosis of specific granules and secretory vesicles. Upon pretreatment with cytochalasin B, these responses are markedly enhanced, and the release of enzymes from the azurophil granules, e.g. elastase, is observed in addition. IL-8 dependent *surface membrane remodeling* during exocytosis leads to the expression of adhesion molecules, CD11b/CD18 (complement receptor type 3), CD11c/CD18 (p150,95) and complement receptor type 1 [4,5]. This upregulation markedly enhances the ability of neutrophils to adhere to endothelial cells and to the extracellular matrix.

The respiratory burst is characteristic for stimulated phagocytes. Like other chemoattractants IL-8 elicits the rapid and transient activation of the NADPH-oxidase, leading to superoxide and H_2O_2 formation. In terms of duration and intensity the respiratory burst induced by IL-8 is considerably weaker than that observed with fMet-Leu-Phe or C5a, but more pronounced than that induced by PAF or LTB_4 [1, 6].

2.3. *Signal transduction*

IL-8 receptors are coupled to *Bordetella pertussis* toxin-sensitive GTP-binding proteins. Signal transduction depends on the activation of a phospholipase C specific for phosphatidylinositol 4,5-bisphosphate, which delivers two second messengers, IP_3 and diacylglycerol. IP_3 induces a rise in cytosolic free calcium, and diacylglycerol activates protein kinase C. ADP-ribosylation of GTP-binding proteins by *pertussis* toxin, depletion of mobilizable calcium, and exposure to protein kinase inhibitors or to wortmannin can be used to modulate signal transduction and to influence shape change, exocytosis and the respiratory burst [7]. The responses of neutrophils to IL-8 and fMet-Leu- Phe are modified in a similar way by such treatments, indicating that they are controlled by a comparable mechanism [8].

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E G A V P L R S A K E L R C Q C . . .   79 aa
    A V P L R S A K E L R C Q C . . .   77 aa
        S A K E L R C Q C . . .         72 aa
            K E L R C Q C . . .         70 aa
                E L R C Q C . . .       69 aa
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Fig. 1. N termini of the naturally occurring, biologically active forms of IL-8.

2.4. *Effects in vivo*

Studies in rabbits showed massive neutrophil accumulation and plasma exudation upon intradermal injection of human IL-8. The effect was rapid and long-lasting. The cellular infiltration was already significant after 15 min, and the immigration of neutrophils remained detectable for up to 10 h [9,10]. Resistance to inactivation and slow clearance from the injection site, possibly due to charge interaction with acidic tissue matrix components, could explain the long duration of action. Comparable responses to human IL-8 were observed in mice, rats, guinea pigs and dogs. Studies in human skin showed the same kind of inflammatory reaction with exclusive infiltration of neutrophils in particular around venules. Lymphocyte numbers were not increased, and no basophils, eosinophils or monocytes were found. IL-8 caused no wheal and flare, itching or pain, suggesting that it does not induce histamine release from skin mast cells [11,12].

3. UBIQUITOUS PRODUCTION

IL-8 was originally identified in the medium of human blood monocytes cultured in the presence of endotoxin, phorbol esters or lectins [1]. It was soon realized, however, that many different cells have the

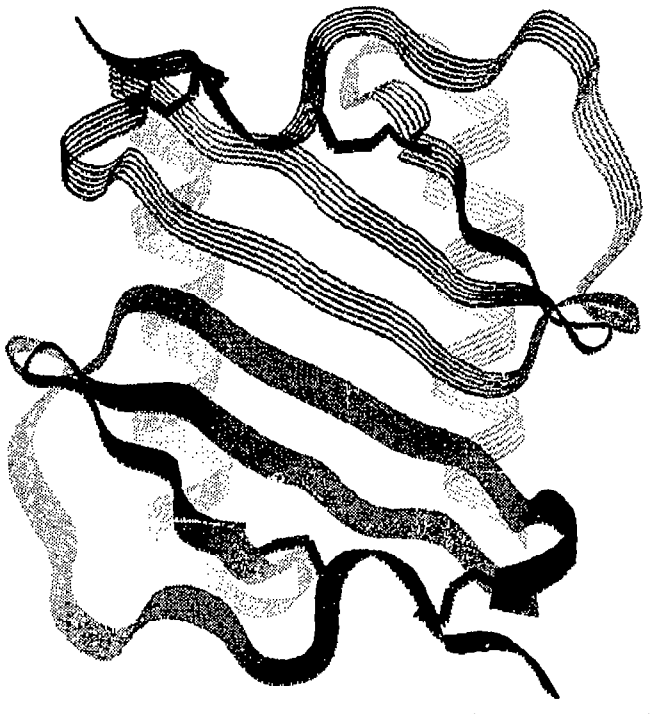

Fig. 2. Ribbon representation of the polypeptide backbone of the 69 amino acid form of IL-8 as a dimer. The N-terminal region (bottom right, top left) is anchored to the rest of the molecule by two disulfide bridges. An N-terminal loop follows the first two cysteines and leads into the central β-strand that interfaces with the other monomer. After two additional β-strands, the structure ends with a C-terminal α-helix. Different shading was used for the monomers.

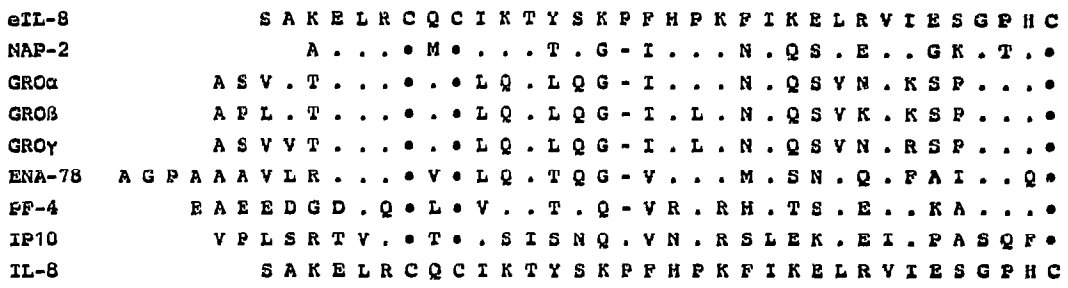

Fig. 3. N-terminal sequences of proteins related to IL-8 (72 amino acid form) aligned according to the cysteines. Note that the ELR (Glu-Leu-Arg) sequence preceding the first cysteine is shared by all proteins with neutrophil-activating properties.

ability to produce IL-8 when appropriately stimulated. The expression of IL-8 mRNA and the release of the biologically active cytokine was observed in endothelial cells, fibroblasts from different tissues, keratinocytes, synovial cells, chondrocytes, several types of epithelial cells as well as some tumor cells [1,13]. Interestingly, even neutrophils can synthesize IL-8 [14], and may thus intensify their own recruitment to sites of inflammation. Interleukin-1 (IL-1) and tumor necrosis factor (TNF) are clearly the most important stimuli, since they were found to induce IL-8 expression and secretion in all cells studied so far [15]. Endotoxin is very effective on phagocytes and endothelial cells, but inactive on mesenchymal cells. Monocytes and macrophages generate IL-8 upon stimulation with IL-1α, IL-1β, TNFα, IL-3, GM-CSF, endotoxin, lectins, phorbol esters, immune complexes and phagocytosis [16]. Opsonized particles are also a prime stimulus for IL-8 production by neutrophils [14].

4. REDUNDANCY

IL-8 belongs to a family of sequence-related 8–10 kDa proteins characterized by the conserved position of their four cysteine residues. Platelet basic protein, connective tissue-activating peptide III and platelet factor 4, which are components of the α-granules of blood platelets, and γ-interferon inducible protein (IP10) were known before IL-8. They differ from IL-8 by their lack of neutrophil-activating properties [1]. Several proteins that share structural and biological similarity to IL-8 were discovered more recently. *Neutrophil-activating peptide*- 2 (NAP-2) derives from the N-terminal processing of platelet basic protein or connective tissue-activating peptide-III, which are released from stimulated platelets [17]. GROα designates a protein that was originally described as melanoma growth stimulatory activity (MGSA) on the basis of its effects on certain melanoma cell lines [18], and was later shown to be a powerful neutrophil chemoattractant [19]. Two related molecules, GROβ and GROγ, were found subsequently [20]. The three GRO proteins have about 90% sequence identity and similar neutrophil-activating properties (Dewald and Clark-Lewis, unpublished results). Finally, an epithelial cell derived neutrophil-activating peptide, ENA-78, was discovered in the cultures of type-II alveolar cells [21].

IL-8 and its five related chemotactic cytokines arise from different genes clustered on chromosome 4 [22]. In pathological conditions, it is likely that several of them are generated concomitantly as suggested by experiments with type-II lung epithelial cells which release IL-8, GROα and GROγ in addition to ENA-78 [21]. It has been shown in several laboratories that the expression of IL-8 and GROα is regulated in a similar way in a variety of different cells [15]. On the other hand, differential expression in dependence of the tissue and the stimulus has been reported for the three *gro* genes [20]. The existence of so many structurally related proteins with comparable neutrophil-activating effects is astonishing. It may be taken to suggest that the function of IL-8 is so important that several back-up genes have evolved. It is conceivable, however, that these different cytokines may have distinguishing biological properties apart from their common effects on neutrophils.

5. IL-8 RECEPTORS

Homologous desensitization was used initially to demonstrate that IL-8 acts via a selective receptor. It was shown that neutrophils remain responsive to IL-8 after stimulation with fMet-Leu-Phe, C5a, PAF or LTB_4, and that IL-8 does not desensitize the cells against these other chemoattractants [23]. IL-8 receptors on human neutrophils and myeloid cell lines were then demonstrated in several independent studies. In general agreement with their results, we have shown that human neutrophils possess on average 64,500 ± 14,000 receptors with an apparent K_d of 0.18 ± 0.07 nM [24]. Competition for the binding of radiolabelled IL-8 was obtained not only with unlabelled IL-8, but also with NAP-2 and GROα. These studies revealed the existence of two types of receptors: one with high affinity for all three ligands (K_d 0.1–0.3 nM), and one with the same affinity for IL-8, but lower affinity for NAP-2 and GROα (K_d 100–130 nM) [24]. Two membrane proteins

that bind IL-8, NAP-2 and GROα were found in crosslinking experiments in agreement with Samanta et al. [25]. Furthermore cross-desensitization indicated that NAP-2 and GROα [24] as well as ENA-78 [21], GROβ and GROγ (Dewald and Clark-Lewis, unpublished results) share the receptors with IL-8.

5.1. *Receptor structure*

A major advance in the understanding of neutrophil activation was the demonstration that chemotactic agonist receptors belong to the seven-transmembrane-domain type. Soon after the pioneering work of Boulay et al. [26] on the fMet-Leu-Phe receptor, two IL-8 receptor cDNAs were cloned and shown to code for similar intramembrane molecules [27,28]. These observations provided the last piece of evidence that IL-8, despite its name, must be regarded as a chemotactic agonist. Extensive biological experimentation had already strongly indicated that the effects of IL-8 on neutrophils were typical for chemoattractants [1], and the recent information on the receptor sequence showed that this interpretation was correct. An important consequence of the finding that IL-8 acts through a chemoattractant, rather than a cytokine, or growth factor-type receptor is that natural antagonists or soluble receptors, analogous to those for IL-1, TNF and some other cytokines [29], are unlikely to be found for IL-8. The cloning of two distinct cDNAs is in agreement with the biochemical evidence for two IL-8 receptors. Information on the sequence of the receptors identified by crosslinking must now be obtained.

5.2. *Structural determinants of receptor binding*

The 3D structure of the IL-8 dimer bears some resemblance with the antigen-binding domain of the HLA molecule. Since the groove that binds the antigen is formed by two α-helices it was thought that the C-terminal helices of the IL-8 dimer constitute the receptor binding site. Recent structure–activity relation studies, however, indicate that IL-8 binds at the N-terminus [30]. Using chemically synthesized analogs, we have found that removal of the entire C-terminal domain decreased, but did not suppress biological activity. In contrast, no receptor binding or neutrophil activation was observed when the N-terminal sequence Glu-Leu-Arg that precedes the first cysteine was deleted. All three residues, Arg in particular, are highly sensitive to modification. The role of Glu-Leu-Arg is also emphasized by a recent mutagenesis study showing that replacement of these residues by alanine results in loss of activity [31]. In this context it is interesting to note that the Glu-Leu-Arg motif is common to all IL-8 related chemotactic cytokines, but is not found in platelet factor 4 and IP10 (Fig. 3). Short peptides containing the Glu- Leu-Arg motif are inactive, suggesting that either a particular conformation of the tripeptide, or interactions of other domains of the IL-8 molecule with the receptor are required. Since nuclear magnetic resonance spectroscopy shows that the N-terminus has a high degree of flexibility, the conformation of the entire protein appears to be important. This is in agreement with the observation that IL-8 is inactivated when the disulfide bonds are eliminated either by reduction or by substitution of the cysteines.

6. PATHOPHYSIOLOGY

The ability to attract and activate neutrophils qualified IL-8 from the beginning as an inflammatory mediator [1]. Meanwhile this hypothesis has been amply verified, in particular by the study of inflammatory skin and joint diseases. Considerable IL-8 immunoreactivity is found in the skin of patients with psoriasis and palmoplantar pustulosis [32–34], where expression of IL-8 mRNA is prominent in keratinocytes [35]. IL-8 accumulates in the synovial fluid of arthritic joints [16,36] and is released by stimulated synovial cells [37] and chondrocytes [38]. Mononuclear cells from the blood or synovial fluid of patients with rheumatoid arthritis release much higher amounts of IL-8 than cells from healthy controls after stimulation with LPS, IL-1, TNF and immune complexes [16].

IL-8 appears to be particularly suited for a role in inflammation and host defence. As a product of different types of cells *it can arise in any tissue* when the levels of IL-1 and/or TNF (which are powerful inducers) are enhanced. Generation of IL-8 can be expected upon infection, ischemia, trauma, and other disturbances of tissue homeostasis, since IL-1 and TNF levels are elevated. In all these conditions IL-8 is likely to be the main cause of the local accumulation of neutrophils [1, 13]. *Resistance to inactivation and slow clearance*, as shown by the long-lasting chemoattractant effect after intradermal injection [10], suggests that IL-8 persists in active form within the immediate environment of the cells from which it is released. Other chemoattractants like fMet-Leu-Phe, C5a, LTB_4 and PAF, by contrast, act more transiently because they are inactivated rapidly by oxidation or hydrolysis [10].

Most remarkable is the *high number of related cytokines*. Five distinct chemotactic proteins have been identified that share structural and biological similarity with IL-8, and bind to the IL-8 receptors. Redundancy strongly suggests that these cytokines are relevant in physiology and pathology. IL-8 and its related cytokines can be expressed concomitantly by cells that are stimulated with IL-1 or TNF. They are likely to concur in recruiting neutrophils, but may also fulfill additional, still unknown functions.

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