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An Interleukin-1/Tumor Necrosis Factor Inducible Inflammatory Cytokine, Interleukin-8

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Chemotactic factors released at foci of injury or infection are thought to mediate directed migration of leukocytes into inflammatory sites. Since the leukocyte composition of the inflammatory infiltrate depends on the temporal stage of the lesion and the nature of the stimulus, it follows that some chemoattractants should be specific for a given type of leukocyte. Although interleukin-1 (IL-1) and tumor necrosis factor (TNF) have been thought to be directly chemotactic for human neutrophils in vitro, we were able to dissociate the major neutrophil chemotactic activity in lipopolysaccharide (LPS)-stimulated human monocyte-conditioned media from IL-1 or TNF [1]. Therefore, we purified the monocyte-derived neutrophil chemotactic factor (MDNCF) to homogeneity, determined the amino terminal amino acid sequence [2] and cloned the cDNA [3]. In this article we will review our recent advances in the biological, biochemical, and molecular biological characterization of this novel chemotactic factor and the study of its receptor. Since MDNCF also chemoattracts T lymphocytes, is biochemically identical to T lymphocyte chemotactic factor (TCF) [4] and is produced by a number of cell types in response to mitogens, LPS, IL-1 or TNF [3], this molecule has been renamed IL-8.

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          1                                                          60
     Hu.F maraalsaap snprlLrVAL LlLLLVaAgr raag..asvA tELRCQClq. TlQgIHPKnI
     Md.G .......... .MtsKLaVAL LaaFLIsAaL cEGavlprsA kELRCQCIKT ySkPfHPKfI
     9e.H .......... .MNgKLg.Av LaLLLVsAaL sqGrtlvkMg nELRCQCIsT hSkfIHPKsI
     Pb.I .......... ......sstk gqtkrnlAkg kEesldsDLy aELRCmCIKT TSg.IHPKnI
     Pf.J .......... .......... .......... .....eaEed gDLqClCVKT TSQ.VrPrhI
     Ip.J .......... .MNqtailic cliFLtlsg. ...iqgvpLs rtvRCtCIsi snQPVnPrsl
Consensus ---------- -MN-KL-VAL L-L-LV-A-L -EG-----LA -ELRCQCIKT TSQPIHPK-I

          61                                                        116
     Hu.F QSvnVkspGP HCaqtEVIAT LKn.GRKaCL nPasPiVKKI IeKMLnsdks N.....
     Md.G keLrVIesGP HCantEIIvk LsD.GRelCL DPkenWVqrV VeKFLkraen s.....
     9e.H QdvkltPsGP HCknVEIIAT LKD.GReVCL DPtAPWVqlI VKaLMakaql NSdapl
     Pb.I QSLEVIgkGt HCnqVEVIAT LKD.GRKICL DPDAPRIKKI VqKkLadeSa d.....
     Pf.J tSLEVIkaGP HCptaqlIAT LKn.GRKICL DlqAPlyKKI IKKLLes... ......
     Ip.J ekLEIIPasq fCprVEIIAT MKkkGeKrCL nPEskaIKnl lKavskemSk rSp...
Consensus QSLEVIP-GP HC--VEIIAT LKD-GRKICL DP-APWVKKI VKKLL---S- NS----
```

Fig. 1. Sequence similarity with other cytokines. Hu.F, human gro, Md.G, MDNCF/IL-8, 9e.H, 9E3; Pb.I, PBP; Pf.J, PF4; Ip.J, Ip 10-gamma. Capital letters show the conserved amino acids.

Sequence Similarity of IL-8 with Other Cytokines and Its Genomic Structure

The deduced amino acid sequence of IL-8 [3] showed striking (40–50%) homology with the deduced sequence of a family of polypeptide factors (8–10 kd) including human platelet basic protein, β-thromboglobulin, platelet factor 4, chicken v-src-inducible protein (9E3), human gamma-interferon inducible protein (IP 10), and a growth-regulated gene product (gro) (fig. 1). The locations of all four cysteine residues are well conserved among these molecules. After our completion of cDNA cloning of IL-8, Schmidt and Weissmann [5] reported an identical cDNA sequence to IL-8 for an inducible molecule in enterotoxin-stimulated human PBMC. Gro has also been identified as a melanoma growth factor by other groups [6]. MIP-2 may be a murine counterpart of IL-8 [7]. There is another group of 8–10 kd proteins consisting of human LD78, RANTES, murine MIP-1, JE, and TCA 3 which are also similar to IL-8 and have significant homology and similarly located cysteine residues.

In order to understand how the IL-8 gene is activated after stimulation with IL-1 or TNF, we cloned the genomic clone of IL-8 (Eco R1-Eco R1, 5.2 kb) from a human placenta genomic library and determined its entire nucleotide sequence [Mukaida and Matsushima, submitted]. The results show that the IL-8 gene consists of 4 exons and 3 introns with a single CAT- and TATA-like structure (fig. 2). The 5′-flanking region of the IL-8 gene shows no overall sequence similarity with that of other cytokines and

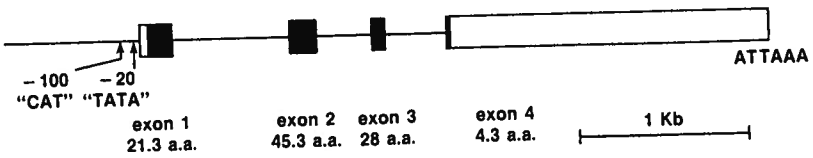

Fig. 2. Genomic structure of IL-8.

polypeptides whose production is also inducible by IL-1 and TNF. The 5′-flanking region, however, contains potential binding sites for several nuclear factors, including activation factor-1, activation factor-2, interferon regulatory factor-1, and hepatocyte nuclear factor-1. In addition, a glucocorticoid-responsive element and heat-shock element were located in the 5′-flanking region. Therefore, the expression of the IL-8 gene may be regulated by the interaction of these factors with the 5′-flanking region. The IL-8 gene is located on human chromosome 4(q12-21) [W.S. Modi et al., unpubl. observation], the site of several other related genes (gro, platelet factor 4 and g-IP 10) [6]. Therefore, these molecules appear to be products of a superfamily of genes. Since it has been reported that a proto-oncogene c-kit, which has structural similarity to other growth factor receptors and has tyrosine kinase activity, is also located in the same region [8] and since some of these molecules can be induced in transformed cells, IL-8 may also have growth promotion activity for some types of cells. However, to date, IL-8 cannot be shown to induce or enhance lymphoproliferation or to promote granulopoiesis.

Induction of IL-8 by IL-1 and TNF

Since both IL-1 and TNF induce neutrophil migration in vivo despite their lack of or limited in vitro chemotactic activity, we hypothesized that IL-1 and TNF might act by inducing IL-8. This hypothesis was tested by examining the induction of mRNA for IL-8 in human monocytes and dermal fibroblasts after stimulation with IL-1 or TNF. One to 100 U/ml IL-1 or TNF induced IL-8 mRNA in both monocytes and fibroblasts within 30 min and maximally at 1–3 h in a dose-dependent manner [3; Larsen et al., in preparation]. These cells also produce IL-8 bioactivity in response to IL-1 or TNF. Keratinocytes and endothelial cells also responded to IL-1 or TNF by expressing IL-8 mRNA [Larsen et al., unpubl. observation]. These data

suggest that numerous cell types produce IL-8 and the in vivo effect of IL-1 and TNF to induce neutrophil migration may be mediated by IL-8 production. When neutralizing antibody to IL-8 becomes available, this hypothesis can be more directly examined.

Biological Activities of IL-8

Both purified TCF and recombinant IL-8 were chemotactic for neutrophils and T lymphocytes in vitro. Injection of recombinant IL-8 into lymphatic drainage areas of lymph nodes in Fisher rats caused accelerated emigration of lymphocytes in high endothelial venules. Intradermal injection of human recombinant IL-8 caused a dose-dependent accumulation of rat lymphocytes and/or neutrophils [4].

IL-8 is not only chemotactic for neutrophils but also induces lysosomal enzyme release and superoxide generation [9, 10]. Intravenous injection of IL-8 caused rapid neutrophilia [11]. IL-8 is chemotactic for human basophils derived from allergic patients and induce histamine release [E. Leonard et al., pers. commun.]. Intradermal injection of IL-8 induces rapid plasma leakage [T. Williams et al., pers. commun.]. Therefore, IL-8 has many of the characteristics of proinflammatory molecules.

Identification and Characterization of the Receptors for IL-8 [12]

Specific receptors for IL-8 have been detected on the surface of human neutrophils and other leukocytes using ^{125}I-labeled recombinant human IL-8. Competitive binding of ^{125}I-IL-8 to human neutrophils reached a maximal level at 1–3 h at 4 °C. Scatchard analysis showed that there are about 20,000 receptors per neutrophil with a single type of high affinity binding ($K_d = 8 \times 10^{-10}$). The receptors for IL-8 are distinct from the receptors for other cytokines and chemotactic agents, such as IL-1, TNF, fMLP, C5a, leukotriene B4 and PAF. Chemical cross-linking of ligand to the receptor followed by SDS-PAGE analysis yielded at least two bands. If one IL-8 molecule binds to one receptor molecule, the MW of the IL-8 receptor on human neutrophil was estimated to be 67,000 and 59,000. Treatment of the human promyelocytic cell line, HL 60 with DMSO to differentiate it into neutrophils increased the number of IL-8 receptors up to 7,000 per cell with a K_d of 1.2×10^{-9} *M*. A low number of receptors for IL-8 was also detected on several other

types of cells including the monocytic cell line, THP-1, and the Epstein-Barr virus transformed B lymphocyte cell line, FMO. The biological significance of the existence of IL-8 receptors on these leukocytes remains to be established. The very low expression of IL-8 receptors on T lymphocytes may reflect that the IL-8 receptor is expressed only on a subpopulation of T lymphocytes.

Conclusion

A novel potent neutrophil and lymphocyte chemotactic activating factor, which is distinct from other cytokines but inducible by IL-1 and TNF, has been purified and molecularly cloned. IL-8 has striking sequence similarity to a group of cytokines, including platelet-derived factors, gro, and gamma-IP10. This molecule has been renamed IL-8. The receptor for IL-8 is distinct from any other known chemotactic factor/agent receptors. The pathophysiological roles of IL-8 in human diseases such as rheumatoid arthritis, asthma, and psoriasis remain to be established.

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