GROWTH FACTORS AND CYTOKINES IN HEALTH AND DISEASE

Editors: *DEREK LEROITH*
CAROLYN BONDY

Volume 1A • 1996

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A Multi-Volume Treatise

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PREFACE

Advances in molecular technology in recent years have catalyzed an explosive growth of information about intercellular peptide messengers and their receptors. For example, ten years ago the only neurotrophin characterized at the molecular level was nerve growth factor (NGF) and the only recognized neurotrophin receptor was the p75 NGF receptor. At present, the number of described neurotrophic peptides approaches 30 and the number of receptors is increasing apace. Just six years ago, the characterized interleukins numbered about three while now there are at least 16. Because many of these new peptide ligands and receptors were identified by "reverse genetic" techniques the understanding of their biological roles lags behind the knowledge of their molecular structures. Over the past few years, however, a new era of functional studies has begun because recombinant proteins have become available for clinical studies. In addition, animal models have been and are being developed using recombinant DNA techniques. Both the clinical studies and studies of transgenic and target deleted mice will allow for further physiologic elucidation of the biological roles of these messenger peptides and their receptors.

This series on Growth Factors and Cytokines is divided into three main sections: Growth Factors (Volume I), Cytokines (Volume II) and Systems (Volume III). Although volumes I and II are separate the distinction between "growth factors" and "cytokines" is probably more historical or pragmatic than indicative of differences in function. The term "growth factors" refers to a wide variety of locally or systemically produced proteins with pleiotropic actions on tissue growth and

differentiation. The term "cytokines" describes a group of proteins identified primarily within the immune and hematopoietic systems, although it is likely that such a narrow view of cytokines will not survive for long. For example it appears that some interleukins and interleukin receptors are expressed by neuroepithelial cells *in vivo* suggesting that these interleukins may have intrinsic roles within the nervous system. Furthermore, tumor necrosis factor (TNF) has been identified as a potential adipose tissue regulatory factor which is both produced and acts locally. The third volume entitled Systems deals more directly with the role of these factors in both normal physiology and the disease processes resulting from the deficiency or excess of growth factors/cytokines and their receptors.

The first volume deals with peptide growth factors and their receptors. Here too there is an arbitrary division of ligands and their receptors. In some instances (e.g., insulin-like growth factors) the proteins and their corresponding receptors are discussed in the same chapter, whereas in other cases, for example, NGF and platelet-derived growth factor they are discussed separately. While we have attempted to be as comprehensive and inclusive as possible, there will always be some regrettable omissions. At the publishing date we recognize that a few growth factors and cytokines have not been included in this review. These new discoveries will for certain be reviewed in similar pages in the future.

Derek Le Roith
Carolyn Bondy

INSULIN-LIKE GROWTH FACTORS

Derek LeRoith and Carolyn Bondy

ABSTRACT

The insulin-like growth factor family of peptides, binding proteins and receptors is involved in normal growth and development. Later they are important in the differentiated function of a number of tissues. Aberrations in this growth factor system are associated with different diseases, ranging from short stature and diabetes to malignancy.

Growth Factors and Cytokines in Health and Disease
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With the advent of recombinant DNA technology, sufficient quantities of the ligands (and binding proteins) have become available for clinical testing in the therapy of certain diseases. These exciting new possibilities need to be assessed carefully for side-effects.

I. INTRODUCTION

The insulin-like growth factors (IGF-I and IGF-II) regulate growth and development of multiple tissues during embryonic and fetal stages (reviewed in Daughaday and Rotwein, 1989; Werner et al., 1994). During postnatal stages they continue to affect growth and maintain the differentiated function in these numerous tissues and in specific cell types. While the liver produces large amounts of both IGFs, many extrahepatic tissues synthesize and secrete these factors as well (Lowe et al., 1987; Hoyt et al., 1988). Circulating IGFs are of hepatic origin and act in a classical endocrine mode, whereas extrahepatic IGFs act locally in a paracrine or autocrine mode. The biological actions of the IGFs are mediated primarily by the type I IGF receptor (IGF-I receptor) which is ubiquitously expressed (LeRoith et al., 1995). The actions of the IGFs are also affected by a family of IGF-specific binding proteins (IGFBPs) found in circulation and in extracellular fluids; these proteins may enhance or inhibit the actions of the IGFs primarily by affecting their availability to cell surface receptors (Baxter and Martin, 1989; Rechler, 1993; Jones and Clemmons, 1995).

In this review we will initially discuss the basic molecular and cellular aspects of the IGFs, their binding proteins and receptors, and use examples from normal physiology and pathology to highlight their importance. Then we summarize the available data on the clinical studies of recombinant human IGF-I (rhIGF-I) and, to a lesser extent, IGF-II which have recently become available for clinical research.

II. MOLECULAR AND CELLULAR ASPECTS

The IGFs are structurally similar demonstrating ~65% amino acid similarity with each other and ~50% with insulin (Blundell et al., 1983; Daughaday and Rotwein, 1989; Sussenbach, 1989; Rechler and Nissley, 1990) (Figure 1). Circulating insulin consists of an A- and B-chain, because the connecting(C) peptide is proteolytically cleaved out during processing of the prohormone. Mature, circulating IGF-I and IGF-II retain the smaller C-peptide and have a D-extension to the A-chain. The E-peptide in the prohormone is cleaved off during processing (see below, Figure 2).

A. IGFs

IGF-I

The human IGF-I gene, on the long arm of chromosome 12 (Tricoli et al., 1984), spans more than 90 kb of chromosomal DNA and contains at least six exons. Exons

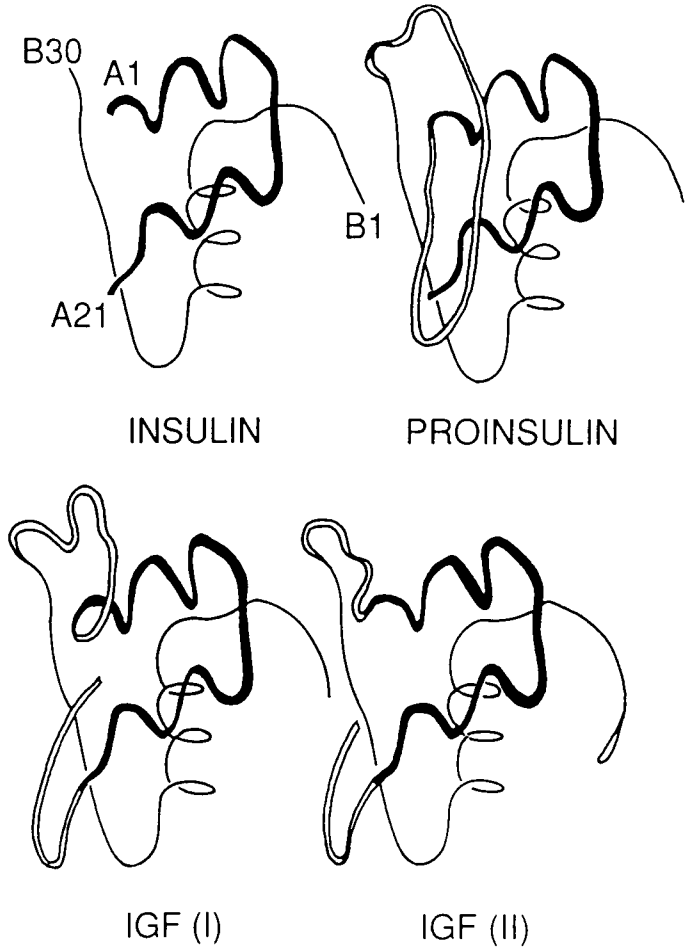

Figure 1. Predicted tertiary structures of the insulin-like growth factor (IGF) family of peptides.

1 and 2 encode distinct, mutually exclusive 5′-untranslated regions (UTRs) as well as distinct N-termini of the signal peptide (Figure 2) (Rotwein et al., 1986; Shimatsu and Rotwein. 1987a). Exons 3 and 4 encode the mature peptide sequence, whereas the E-peptide coding sequences are contained in exons 4, 5 and 6.

Transcriptional and posttranscriptional events are extremely complex. For example, the exon 1 promoter lacks core promoter elements, such as TATA and CCAAT boxes, and transcription of this exon is, therefore, initiated from at least four sites dispersed over a ~350 bp region. Transcription from exon 2, which contains TATA- and CCAAT-like motifs, is initiated over a smaller region (Jensen et al., 1991).

Exon 1-containing transcripts are expressed ubiquitously, and transcription is regulated by multiple factors, generally, specific to each particular tissue. Exon 2-

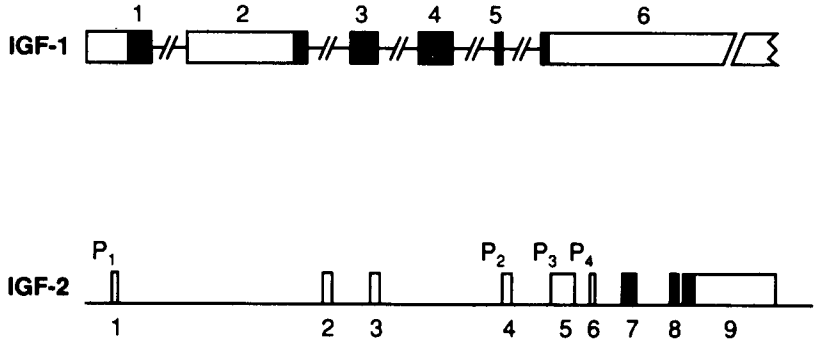

Figure 2. Structure of mammalian IGF-I and IGF-II genes. Exons are numbered. Known promoter sites in the IGF-II gene are labeled P1–P4.

containing transcripts, on the other hand, are particularly abundant in the liver and are generally more responsive to growth hormone (GH). Thus, during development, exon 2-containing transcripts appear after exon 1-containing transcripts in the liver, but increase markedly at the onset of GH-dependent linear growth (Jensen et al., 1991; Kim et al., 1991; Kikuchi et al., 1992).

At the posttranscriptional level there appears to be regulation of mRNA splicing, in certain species of IGF-I mRNA (Shimatsu and Rotwein, 1987b), of a 186 bp region of exon 1 that potentially influences translatability of that transcript. Furthermore, two alternative E-peptides may be transcribed depending on exon 5 or exon 6 usage; GH seems to favor exon 5 retention. At the level of mRNA stability, the longer ~7.5 kb transcripts, derived from distal polyadenylation site usage in the long exon 6, are more unstable than the shorter ~1 kb mRNAs derived by more proximal polyadenylation site usage (Lowe et al., 1988; Lund et al., 1989; Heppler et al., 1990; Steenbergh et al., 1991).

IGF-II

The IGF-II gene spans ~30 kb of chromosomal DNA on the distal end of the short arm of human chromosome 11 (Tricoli et al., 1984), immediately 3′ to the insulin gene. Like IGF-I, the IGF-II gene is complex consisting of nine exons. The mature peptide is encoded by exons 7, 8 and 9. Transcription is controlled by four different promoters (P1 – P4) (Dull et al., 1984) (Figure 2). The promoters are activated in a tissue- and development-specific manner; promoter P1 is activated in adult liver, whereas promoters P2, P3 and P4 are active in most fetal tissues and adult nonhepatic tissues (dePagter-Holthuizen et al., 1987; dePagter-Holthuizen, 1988; Holthuizen et al., 1990). P1 is a TATA-less, GC-rich promoter with heterogeneous transcription initiation. Liver-specific expression of P1 is regulated by the CAAT/enhancer binding protein (C/EBP) and the liver-enriched activator protein

(LAP) (Sussenbach et al., 1994). Promoters P3 and P4 contain a TATA box and P3 also has a CCAAT box. P3 and P4 exhibit transcription from specific sites and are more highly regulated than P1. Human IGF-II promoter P3 is expressed in many fetal and non-hepatic adult tissues and is regulated by the krox 20/egr2 and krox 24/egr1 transcription factors (Sussenbach et al., 1994). Multiple IGF-II mRNA transcripts are produced as a function of specific promoter usage and different lengths of 3′ UTRs resulting from use of multiple polyadenylation sites. Transcripts from P2 and P4 have shorter 5′-UTRs and are preferentially translated (Irminger et al., 1987; Rechler, 1991).

In human tissues, the IGF-II gene demonstrates promoter-specific genomic imprinting (Vu and Hoffman, 1994). When promoter P1 is used, as with adult liver, both maternal and paternal alleles are transcribed, but in the case of promoters P2–P4 usage, only the paternal allele is expressed. Because promoters P2–P4 are clustered in a ~5 kb DNA region, whereas P1 is ~20 kb upstream (Figure 2), this suggests that the imprinting signals lie between promoters P1 and P2 (Vu and Hoffman, 1994).

In murine species, the equivalent of promoter P1 is absent and the remaining three promoters are all imprinted except for the choroid plexus and leptomeninges where biallelic expression occurs (Pedone et al., 1994; DeChiara et al., 1991a; DeChiara et al., 1991b). Interestingly, in the mouse and rat, postnatal IGF-II expression decreases dramatically leading to undetectable circulating IGF-II levels (except in the CNS), whereas in humans, where the P1 promoter is active in liver, IGF-II expression persists throughout life. Because the P1 promoter is biallelically expressed, it has been postulated that this may allow more abundant synthesis of the IGF-II peptide which is released into the circulation. Loss of genomic imprinting has been implicated as a reason for the high level of expression of IGF-II in many cancers (Steenman et al., 1994; Werner and LeRoith, 1995), where the IGF-II peptide may be important as an autocrine growth factor enhancing tumor growth (Zhan et al., 1994).

B. Receptors

The biological effects of the IGFs are mediated by a family of specific membrane-associated glycoprotein receptors including the insulin IGF-I and IGF-II receptors and the insulin receptor-related-receptor (IRR) (Zhang and Roth, 1992; Reinhardt et al., 1993; Kovacina and Roth, 1995; LeRoith et al., 1995). The insulin IRR, and IGF-I receptors are closely related tyrosine kinase receptors, whereas the IGF-II receptor is identical to the cation-independent mannose-6-phosphate (M-6-P) receptor (Figure 3). Because the biological effects of the IGFs on growth, development, and differentiated functions are primarily via the IGF-I receptor, we will concentrate on its structural and functional characteristics. Because the IRR has limited tissue distribution and does not bind the IGFs, it does not apparently affect IGF action.

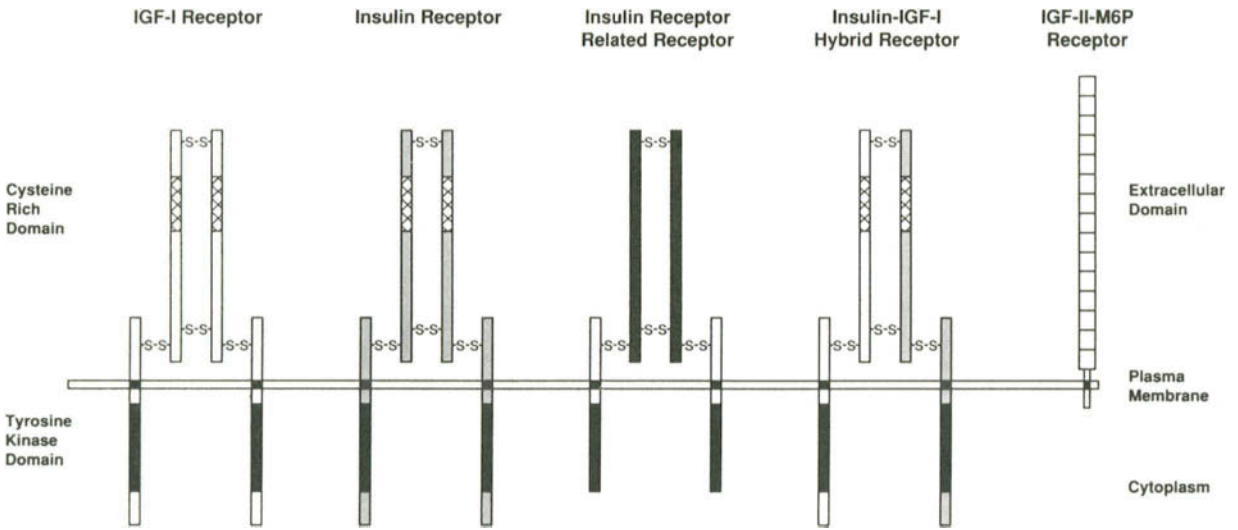

Figure 3. Comparison of IGF-I, insulin, insulin receptor-related receptor, insulin-IGF-I hybrid, and IGF-II/M-6-P receptors. The biological actions of insulin and the IGFs are initiated by their interaction with specific cell surface receptors. Insulin and IGF-I receptors are tetrameric proteins composed of two extracellular α subunits and two transmembrane β subunits. The insulin receptor-related receptor shows limited tissue expression. Naturally occurring hybrid receptors have been described in which an insulin α/β hemireceptor is linked to an IGF-I α/β hemireceptor. The IGF-II/M-6-P receptor is a single chain polypeptide comprising 15 repeat sequences and a short cytoplasmic domain.

IGF-I Receptor

The human IGF-I receptor spans more than 100 kb of chromosomal DNA at the distal end of the long arm of chromosome 15 and consists of 21 exons (Abbott et al., 1992). The gene encodes contiguous α and β subunits which are cleaved during processing and the mature functional receptor is a heterotetrameric glycoprotein in an $(\alpha\beta)_2$ configuration where the subunits are joined by disulfide bridges. The α subunits are entirely extracellular and bind the ligands, primarily, in the region of the cysteine-rich domain. The β subunits are anchored in the membrane and contain a cytoplasmic tyrosine kinase domain which has 84% homology with the equivalent region in the insulin receptor and IRR (Ebina et al., 1985; Ullrich et al., 1985; Ullrich et al., 1986).

The IGF-I receptor is widely expressed at high levels during embryogenesis, suggesting an important role in tissue development (see below). Extensive studies on characterizing the promoter region have revealed a number of features involved in regulating IGF-I receptor gene expression (Werner et al., 1989; Werner et al., 1990; Cooke et al., 1991; Mamula and Goldfine, 1992; Werner et al., 1992). Both the unusually long (~1 kb) 5′ UTR and the proximal ~500 bp 5′ flanking region are very GC-rich with multiple Sp1 binding sites and no TATA or CCAAT boxes. However, transcription initiation begins at a single site surrounded by an "initiator" sequence. Sp1 binding to the promoter region may regulate transcription initiation in the absence of TATA and CCAAT boxes (Smale and Baltimore, 1989).

Ligand binding to the IGF-I receptor initiates receptor autophosphorylation which involves the phosphorylation of a cluster of three tyrosines (1131, 1135 and 1136) in the kinase domain (Gronberg et al., 1993; Kato et al., 1993; Kato et al., 1994). As with the insulin receptor, autophosphorylation activates the tyrosine kinase activity of the receptor, leading to tyrosine phosphorylation of cellular substrates. The substrate that has been best characterized to date is the insulin substrate-1 (IRS-1) (Sun et al., 1991; Myers and White, 1993) (Figure 4). IRS-1 contains multiple tyrosine residues in a Tyr-Met-X-Met (YMXM) or related motifs. Phosphorylation of tyrosines in these motifs mediate binding of other substrates, such as the 85-kDa subunit of phosphatidyl-inositol 3′ (PI3′) kinase and Grb2 (Myers et al., 1992; Yamamoto et al., 1992; Baltenspergan et al., 1993; Giorgetti et al., 1993; Myers et al., 1993; Skolnik et al., 1993). IGF-I stimulates PI3′ kinase activity and MAP kinase activity, the latter being the result of activation of the *ras/raf* kinase pathway initiated by Grb2 binding to IRS-1. Other known pathways involved in IGF-I receptor activation include protein tyrosine phosphatase-1B (Kenner, 1993) and SHC and crk (Beitner-Johnson and LeRoith, 1995).

IGF-II/M-6-P Receptor

The IGF-II/M-6-P receptor is a bifunctional receptor with a large extracellular region containing 15 contiguous repeats and a very short cytoplasmic tail (Lobel et al., 1988; MacDonald et al., 1988; Morgan et al., 1987). Unlike the insulin and