Insulin-Like Growth Factor-I: Compartmentalization Within the Somatotropic Axis?

Andrew A. Butler,1 Shoshana Yakar,2 and Derek LeRoith2

1The Vollum Institute, Oregon Health Sciences University, Portland, Oregon 97201; and 2Clinical Endocrinology Branch, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892-1758

Insulin-like growth factor-I (IGF-I) is essential for normal growth; igf-1 gene mutations are associated with extreme growth retardation in mice and, very rarely, in humans. The relative contributions of tissue vs. endocrine (hepatic) IGF-I to the regulation of growth have been a fundamental question. New gene targeting technologies are providing answers for these questions.

The growth of an organism is dependent on the integration of internal signals, which ultimately stem from the program driven by the genome, with environmental cues. Nutrition is an obvious environmental factor over which the organism has no influence, but the organism must be able to respond to it to produce a viable adult. Temperature is another important variable, with the increased metabolic demands of low temperatures leading to changes in the size of certain organs. The organism must also determine when to stop growing and commit resources to reproduction (i.e., puberty). Flexibility of the genetic program is therefore a requirement, with growth resulting from the integration of the constraints imparted by the environment with the genetic program.

It has been known for over half a century that the primary factor controlling growth rate is growth hormone (GH) (8). GH was originally believed to stimulate growth via the stimulation of an intermediary, somatomedin, which is secreted by the liver and acts as a circulating growth factor (4). The isolation and sequencing of the genes and cDNAs of the insulin-like growth factors (IGF) led to the discovery that, although the livers of adult mice had by far the highest level of expression, IGF-I mRNA expression was essentially ubiquitous. IGF-I mRNA was also found to be expressed in the fetus, a stage in development at which GH does not appear to play a significant role in regulating growth (3). The question thus raised was: what are the respective roles of endocrine IGF-I, primarily hepatic in origin, and IGF-I synthesized in the peripheral tissues and presumably acting in an autocrine/paracrine manner? This review will provide a brief introduction to the somatotropic axis and will discuss some of the recent developments made possible by the use of modern genetic techniques.

Overview of the somatotropic axis

GH is secreted from somatotrophs, specialized cells present in the anterior pituitary, and represents the leading candidate for the apical “growth regulator.” Modern genetic analysis has shown that dwarfism in humans can result from mutations leading to loss of GH secretion, such as, for example, mutations in the GH-releasing hormone (GHRH) receptor, in the GH gene itself, in transcription factors necessary for GH gene activity (e.g., Pit-1), or in the GH receptor (GHR, “Laron Dwarfism”) (8). However, the observation of rare cases in which normal growth is observed to be associated with normal serum IGF-I levels in the absence of pituitary GH suggests that our understanding of growth regulation is not complete.

GH activity is regulated at several levels, with both the magnitude and pattern of GH secretion influencing the biological response (8). The secretion of GH from pituitary stores has long been known to be regulated by the interplay of two hypothalamic neuropeptides. GH release is stimulated by GHRH and is inhibited by somatostatin. The pattern of GH secretion is an important determinant of growth rate, with a discrete pulsatile secretory pattern stimulating growth more efficiently than continuous infusion. The pattern of GH release is sexually dimorphic. Males exhibit discrete pulses with low interpeak concentration, whereas females tend toward a high basal level of GH and less evidence of pulsatility. GH activity can be regulated at the level of the GHR, with malnutrition in particular associated with a state of GH resistance.

IGF-I is also essential for normal growth, with the original gene targeting studies in the mouse indicating that IGF-I is important for pre- and postnatal growth (1, 5). Confirmation of the significance of IGF-I in human physiology was obtained by the discovery of a patient with intrauterine growth retardation and postnatal growth failure associated with a mutation in the igf-1 gene (11). Mice lacking a functional igf-1 gene exhibit growth retardation both in utero and postnatally, whereas mice lacking a functional igf-2 gene exhibit intrauterine growth retardation but have normal growth velocity postnatally. This is consistent with the developmental profile of IGF-I and IGF-II mRNA expression in the mouse and rat, in which IGF-II mRNA expression dramatically declines in the first 10 days postpartum. However, an important caveat is that the profile of IGF expression varies considerably between species. In other mammalian species, including humans, IGF-II expression and presence in the circulation persists into adulthood. The function of IGF-II in the adult of nonrodent mammals remains an enigma, because there have been no reports of naturally occurring igf-2 mutants.

IGF-I and its receptor (IGF-IR) belong to a family of peptides related in structure to insulin and its receptor (Fig. 1). Two IGF peptides have been isolated that exhibit distinct development and tissue-specific patterns of expression. Both IGF-I and IGF-
II activate IGF-IR, and IGF-II is also an agonist of a splice variant of the insulin receptor that is expressed at high levels in fetal and neoplastic tissues. A third receptor, the mannosyl-6-phosphate/IGF-IR, binds IGF-II with high affinity and appears primarily to function as a "biological sink" for IGF-II, increasing clearance and degradation.

**The liver is the primary source of circulating IGF-I**

Several observations supported the contention that IGF-I in the circulation is primarily hepatic in origin. In rodents, IGF-I mRNA expression increases dramatically in the liver during the first 50 days of life. The increase in expression ensures that, in the young adult, IGF-I mRNA is approximately two orders of magnitude more abundant in liver than other tissues. The rise in hepatic production coincides with an increase in IGF-I in the circulation, while hepatic IGF-I mRNA exhibits the most dramatic response to GH depletion and replenishment.

A recent publication reports that serum IGF-I levels in mice lacking a functional GHR could be as low as 0.2% of normal levels (7). A common finding in mutant mice in which either GH or the GHR genes are compromised is the dramatic decline in IGF-I mRNA in liver, accompanied by smaller declines in some tissues (e.g., kidney, ovary, muscle) and some tissues in which IGF-I expression is not affected (e.g., lung, heart, testis, brain). Indeed, this has led Lupu and colleagues (7) to suggest that circulating IGF-I is exclusively hepatic in origin and that the residual levels observed in mice in which the igf-1 gene has been targeted for excision by the Cre-loxP system, as discussed below, is perhaps due to incomplete recombination. However, it could also be argued that IGF-I mRNA expression is not necessarily correlated with translation. Measurements of IGF-I protein in the tissues of the GHR knockout in which IGF-I mRNA expression is not reduced will be needed to confirm that posttranscriptional steps of IGF-I synthesis have not been affected.

**Interaction between GH and IGF-I in regulating growth**

Given the level of interest in the field concerning the respective roles of GH and IGF-I in growth regulation, it is perhaps surprising that the first study of ghrligf1 knockout mice has only recently been published (7). This study has confirmed that GH and IGF-I have independent and common functions. Double ghrligf1 nullizygotes exhibit a more severe phenotype than either the igf1 or ghrl knockout. This study provides an interesting comparison of the respective roles of GH, signaling through the GHR, and IGF-I in murine growth, which is summarized in Table 1. The study confirms several previous reports that GH does not appear to significantly affect growth until late in the second week postpartum, with the most dramatic differences in growth velocity between GH-sufficient and GH-deficient mice occurring between postnatal days 20 and 40.

Significantly, the growth rates of double ghrligf1 nullizygotes are not significantly different from those of igf1 nullizygotes until 2 wk postpartum, at which time the double mutants began to exhibit a more significant growth retardation. Ultimately, adult ghrligf1 nullizygotes weigh ~17% of normal, whereas igf1 nullizygotes weigh ~30% of normal. Thus ~83% of the final weight of a mouse is due to the growth-stimulating actions of the somatotrophic axis. The authors use the growth curves of the single and double knockouts to estimate the contribution of GH and IGF-I separately and in synergy with the 83% of growth due to the somatotropic axis. They suggest that ~35% of the final weight of a mouse is due to IGF-I action, 14% is due to GH acting independently of IGF-I, and the GH-IGF-I interaction contributes 34%. Note that these figures represent approximations for the contribution of GH and IGF-I in the background of the mouse strains used for the study.

**Tissue-specific knockouts to examine the role of hepatic IGF-I**

Recently there has been an advance in in vivo gene targeting studies allowing the study of the function of genes in either a tissue- or temporal-specific manner in the whole animal. The ability to inactivate genes in a tissue- and time-regulated manner represents an incredibly powerful new tool for the molecular biologist. Genes may be excised from the genome by using a site-specific recombination technology adapted from bacteriophage. Bacteriophage P1 Cre recombinase, a 38-kDa protein, recognizes 34-bp DNA sequences called locus of crossover P1 (loxP) sites (2, 9). When the loxP sites are in tandem and flanking an essential exon of the gene of interest, Cre induces an intramolecular recombination and excision of the intervening DNA, resulting in deletion of the exon. By placing the Cre recombinase under the control of tissue-specific or inducible promoters, the function of a gene in a certain tissue(s) can be examined in the absence of poten...
tially critical developmental abnormalities. Mice with a gene flanked by loxP sites therefore represent an extremely valuable resource.

By using homologous recombination, a mouse line has been established in which two loxP sites in tandem flank the fourth exon of the IGF-I gene (6, 10, 13). Exon 4 encodes amino acid residues 26–70 of the IGF-I peptide, including part of the B domain and the entire C, A, and D domains. This region of the peptide is solely responsible for IGF-I binding to its cognate receptor (IGF-IR) and has been previously targeted by others to create a total IGF-I knockout for developmental studies (1). To create mice with targeted inactivation of the IGF-I gene in the liver, transgenic mice that express Cre recombinase exclusively in the liver by expressing Cre under the control of the albumin promoter were generated (13). The albumin gene is activated in hepatocytes during fetal development, with transcripts becoming easily detectable at ~10 days postnatally. Cross-breeding of the loxP-flanked IGF-I mice and the albumin-Cre-expressing mice resulted in deletion of the IGF-I gene in the liver. As assayed by Southern blot analysis, the IGF-I gene deletion effect in liver was ~95%. In other tissues, there was no evidence of recombination. Analysis of IGF-I mRNA expression by solution hybridization RNase protection assay confirmed that the targeting had worked, with liver IGF-I mRNA levels reduced to <1% of wild-type expression levels. IGF-I mRNA levels measured in nonhepatic tissues of the liver-specific knockouts such as heart, muscle, fat, spleen, and kidney, IGF-I mRNA levels were not different from wild-type levels. From these results, we have concluded that these tissues do not show compensation, although we cannot exclude the possibility that compensation could occur at the level of IGF-I translation.

At this stage it is still unclear whether the normal growth and development in these mice is due entirely to local IGF-I production or whether the free circulating IGF-I levels are sufficient to maintain this function. This latter possibility could explain why when IGF-IR mRNA levels were measured in various tissues they were not different from those in wild-type mice. Alternatively, free IGF-I levels are normal (10) and could be sufficient to maintain endocrine IGF-I-induced growth. However, it is clear that no compensation is seen with IGF-II, because IGF-II mRNA levels in tissue and IGF-II protein levels in serum were both undetectable in the IGF-I gene-deleted mice.

Insulin resistance in muscle of liver-specific igf-1 knockouts

The increase in circulating GH consistently observed in liver-specific igf-1 knockouts could potentially have important physiological consequences (10, 13). Insulin resistance is a

![FIGURE 2. Deletion of the liver IGF-I gene results in elevated serum growth hormone (GH) levels due to the reduced circulating IGF-I levels. This is associated with insulin resistance, primarily in the skeletal muscle, and with secondary hyperinsulinemia, which may also be caused by GH-stimulated islet cell hyperplasia.](http://physiologyonline.physiology.org/)

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<tr>
<th>Period/Predominance</th>
<th>IGF-I</th>
<th>GH and IGF-I</th>
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<tbody>
<tr>
<td>0–10 days</td>
<td>60</td>
<td>40</td>
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<td>3–7 wk</td>
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<tr>
<th>IGF-I knockout</th>
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<td>Ghr knockout</td>
<td>Normal</td>
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<td>Double knockout</td>
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Values are % of normal. Note that mice lacking a functional ghr gene have normal body weight at birth and do not show signs of growth retardation until 3 wk of age. In contrast, mice lacking a functional igf-1 gene exhibit growth retardation throughout all stages of development.
common feature of states in which GH levels are chronically elevated, and a recent study has confirmed that a state of insulin insensitivity exists in liver-specific igf-1 knockouts (12). Liver-specific igf-1 knockouts are mildly hyperinsulinemic and are resistant to the glucose-lowering effects of exogenous insulin, but they clear a glucose load normally. Insulin-induced phosphorylation of the insulin receptor and insulin receptor substrate-1 is absent in muscle but is normal in liver and white adipose tissue. Chronic treatment with IGF-I reduced circulating GH to normal levels and increased insulin sensitivity. Treatment with a GHRH antagonist, which reduced GH levels, also increased insulin sensitivity.

This study also showed that the levels of “free” IGF-I were normal in liver-specific igf-1 knockouts. The insulin insensitivity thus appears to be a deficit associated with mild hyposomatropism. An interesting question yet to be answered is why GH levels are elevated despite unbound IGF-I, which presumably represents the biologically active fraction of IGF-I in the circulation, being normal. Nevertheless, it appears that a major role of IGF-I in the circulation is to promote insulin sensitivity in muscle, perhaps indirectly via a feedback effect on GH secretion (Fig. 2).

Conclusions

Recent studies suggest that the majority of growth (83%) in the mouse is the result of the somatotropic axis. The contribution of the various components of the axis (GH, IGF-I) varies considerably between developmental stages. IGF-I stimulates growth throughout development, whereas GHR-dependent growth does not develop until the second week of life. The growth-promoting effects of IGF-II, which have not been discussed in this review, are limited to growth in utero in the mouse. The concept of separate endocrine vs. paracrine compartments of IGF-I is supported by the observation that GHR mutants have no measurable IGF-I in the circulation but have normal expression of IGF-I mRNA in peripheral tissues. Confirmation that IGF-I production is truly GH independent in peripheral tissues will require the measurement of IGF-I protein in these tissues in the GHR knockout and also in models such as the liver-specific IGF-I knockout. Studies examining the function and regulation of IGF-I will be greatly aided by the development of tissue-specific knockouts of the IGF-I and IGF-IR genes.

References