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Editorial: Insulin-Like Growth Factor (IGF)-Binding Proteins in Serum—Do They Have Additional Roles besides Modulating the Endocrine IGF Actions?

Although the existence of insulin-like growth factor (IGF)-binding proteins (IGFBPs) in serum was suspected over 20 yr ago, it is only recently that we have begun to gain insight into the biological significance of the various IGFBPs. Six IGFBPs, designated IGFBP-1 through IGFBP-6 (24–50 kDa in size) have been found in serum, tissue extracts, and culture medium that was conditioned by a variety of cell types. The structural features that distinguish one IGFBP from another include an RGD cell matrix adhesion sequence, glycosylation sites, phosphorylation sites, and extracellular matrix binding (1, 2). In this editorial, we discuss the possible reasons for the redundancy in IGFBPs (why six IGFBPs instead of one?) and evaluate the potential roles that IGFBPs may play in regulating the endocrine and local actions of the IGFs.

Endocrine and local actions of IGFs

In adult humans, the total concentration of IGF-I and IGF-II in blood is about 800 μg/L or 0.1 μmol/L, which is approximately 1000 times greater than the insulin concentration (3). Despite the fact that the insulin-like activity of IGFs is only 5% that of insulin, the IGFs could in theory contribute 50 times more insulin-like activity than insulin alone because of their abundance. However, this does not occur because the activity of IGFs is largely neutralized as a consequence of binding to the IGFBPs. Thus, without IGFBPs, the effect of IGFs would swamp any effect of insulin alone, and because IGF expression is not tightly controlled by blood glucose levels, glucose homeostasis would not be possible.

One obvious question that arises with regard to the circulating IGF concentration is why are the IGFs present in such abundance compared to other polypeptide growth factors in blood? Because the circulating level of the IGFs is largely determined by GH and nutrition and because IGFs have been shown to stimulate the growth and differentiation of a number of cell types, it is interesting to speculate that circulating IGFs may function to promote general growth. Thus, the reason why IGFs may circulate at high levels in blood, is so that a large readily available reserve is available for IGFs to act systematically in an endocrine manner. In addition to general systemic regulation, there is also a need for local regulation of growth in specific organs depending on local needs. For example, mechanical loading at a local site in bone stimulates local bone growth and not generalized skeletal growth. The current evidence strongly suggests that IGFs may play a significant role in such a local regulation inasmuch as IGFs produced by one cell type act on the same cell in an autocrine manner or on a neighboring cell type in a paracrine manner (3). Thus, IGFs may function locally in a variety of cell types, including brain, muscle, kidney, lung, pancreas, and bone, in an autocrine/paracrine manner in addition to their systemic endocrine actions.

Tissue-specific regulation of IGF actions

If IGFs play a central role in the local regulation of a number of tissues, then how are the actions of IGFs regulated differently in various tissues? This is obviously an important question because IGFs are produced by most cell types, but their role in the regulation of cell proliferation and other functions appears to vary from one tissue to another. Thus, some means must exist for IGF signal molecules, which are common to so many tissues, to act on a given tissue in a specific manner. One means by which tissue specificity can be accomplished is by producing multiple forms of a given growth factor. For example, the fiboblast growth factors, the interleukins, and the transforming growth factor-β (TGFβ) groups of growth factors each contain several members, some of which have been shown to function in a tissue-specific manner. In the case of IGFs, only two forms of IGFs are produced by many tissues. However, tissue-specific regulation may be accomplished partly by the presence of multiple forms of IGFBPs and their corresponding proteases. For IGFBPs to serve as efficient tissue-specific regulators of IGF activity, it can be predicted that multiple components will be required to serve multiple effector inputs from different tissues. In this regard, the following findings support the concept that the six IGFBPs and their corresponding proteases (unknown number) may provide a mechanism for tissue-specific regulation of IGF actions: 1) IGFBPs differ in their biological actions depending on the cell type studied; 2) IGFBPs are differentially expressed in different tissues in both fetal and adult life; and 3) extracellular fluids of certain tissues are enriched with specific IGFBPs (*e.g.* IGFBP-1 in human amniotic fluid, IGFBP-6 in human cerebrospinal fluid, and IGFBP-3 in human follicular fluid) (1, 2, 4).

IGFBPs as potential modulators of endocrine IGF actions

In the circulation an, about 75% of the IGFs are complexed with IGFBP-3 and an acid-labile subunit in a 150- to 200-kDa ternary complex, whereas the remainder of the circulating IGFs are bound to lower molecular mass IGFBPs (5–7). With the recent development and validation of RIAs for accurate measurements of various IGFBPs in biological fluids, it is

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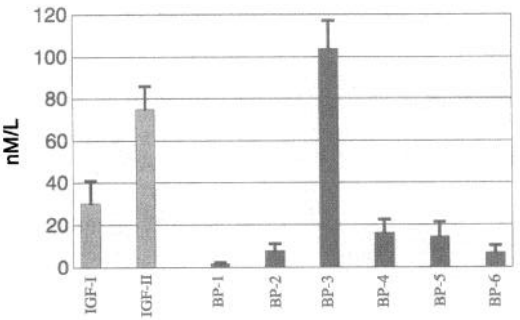

FIG. 1. Concentrations of IGFs and IGFBPs in adult human serum. IGF-I, IGF-II, IGFBP-3, IGFBP-4, and IGFBP-5 values were measured by RIA in the author's laboratory. Data for IGFBP-1, IGFBP-2, and IGFBP-6 were compiled from published literature. Values are the mean ± SD.

now possible to evaluate the relative contributions of all six IGFBPs to the IGF-binding capacity of serum (Fig. 1). The total concentration of the small molecular mass IGFBPs (IGFBP-1, -2, -4, -5, and -6) add up to about 50% of the IGFBP-3 concentration (8–18). Based on the observation that there is a 50% molar excess of IGFBPs over IGFs, it is unlikely that there is much free IGFs in normal adult human serum.

If the majority of the IGFs circulate in serum as IGF-IGFBP complexes, it should be possible to modulate the endocrine actions of IGF by several mechanisms, including those that regulate the levels of IGFBPs (Table 1). In this regard, there is evidence that the relative concentrations of various IGFBPs in the serum can change depending on physiological and pathological situations, such as age, nutrition, serum GH levels, diabetes, puberty, pregnancy, *etc.* (1, 8–18). Studies of serum regulation of various IGFBPs to date reveal that the candidate IGFBP chosen as a primary regulator of IGF bioavailability may vary depending on the metabolic conditions. For example, IGFBP-1 appears to be the primary regulator of IGF bioavailability in response to acute changes in the circulating insulin level, whereas IGFBP-3 appears to be the primary regulator of IGF bioavailability in response to changes in the circulating GH level (5). In addition, serum levels of IGFBP-5, but not IGFBP-4, showed significant positive correlations with IGF-I and IGF-II, suggesting that different mechanisms may regulate the amount of inhibitory IGFBP-4 and stimulatory IGFBP-5 in human serum (8, 9). Although the findings that serum levels of one or more IGFBPs change acutely upon treatment with a number of physiological regulators and that they change in the right direction to explain the corresponding metabolic change are consistent with the general hypothesis that the endocrine IGF actions may in part be regulated by changes in serum IGFBP levels, further studies are needed to establish the cause and effect relationship.

As the IGF system in serum is made up of multiple components, it would be important for the stimulatory and inhibitory components of the IGF system to be regulated in a reciprocal manner to produce an optimal effect in response to changes in physiological or pathological conditions. Otherwise, parallel changes in inhibitory and stimulatory components of the IGF system would cancel each other out. An example of a situation in which a coordinated regulation of multiple IGF system components occurs can be seen during aging (8–18). With advancing age, not only does the concentration of IGFs decrease, but the concentration of stimulatory IGFBP-5 decreases, whereas the concentrations of inhibitory IGFBP-1, IGFBP-2, and IGFBP-4 increase with age (Table 2). Thus, the multiple deficits in the IGF system components that occur as a function of age lead to a marked decrease in the ratio of stimulatory IGFs to inhibitory IGFBPs, which would tend to decrease the endocrine actions of IGFs.

TABLE 1. Potential mechanisms to increase IGF bioavailability in the target tissues

1) Increase the level of free IGFs
2) Increase the ratio of IGFs/inhibitory IGFBPs
3) Increase the ratio of stimulatory IGFBPs/inhibitory IGFBPs
4) Increase the rate of proteolysis of inhibitory IGFBPs in the target tissue
5) Decrease the rate of proteolysis of stimulatory IGFBPs in the target tissue
6) Increase IGF receptor abundance in the target tissue

TABLE 2. Age-related changes in IGF system components

Component	Function	Change (%)
IGF-I	Stimulatory	60 ↓
IGF-II	Stimulatory	15 ↓
IGFBP-1	Inhibitory	20 ↑
IGFBP-2	Inhibitory	35 ↑
IGFBP-3	Stimulatory/inhibitory	25 ↓
IGFBP-4	Inhibitory	35 ↑
IGFBP-5	Stimulatory	35 ↓
IGFBP-6	Inhibitory	?

Estimated percent change between 20–29 and more than 60 yr of age (see text for references).

Another potential control mechanism for regulation of endocrine IGF actions is by IGFBP-specific proteases. Since the discovery of IGFBP proteases in pregnancy serum several years ago (19, 20), scores of papers have been published identifying the IGFBP protease activities in the conditioned media of various cell types and in sera or body fluids of patients with a variety of pathophysiological conditions (21–25). Although it is not known at this time whether the various IGFBP proteases identified are absolutely specific to IGFBPs or whether there are multiple proteases for each IGFBP, the findings that the rate of IGFBP proteolysis can be regulated by a variety of systemic and local effectors raise interesting possibilities for the involvement of IGFBP proteases in regulating the endocrine actions of IGF. For example, IGFBP-3 protease has been proposed to play an important role in modulating the bioavailability of IGFs. As the ternary complex of ALS-IGFBP-3-IGF-I does not appear to cross the vascular endothelium, it has been proposed that an increase in IGFBP-3 proteolysis leads to the breakdown of the ternary complex and a corresponding increase in the formation of a lower molecular mass IGF-IGFBP complex, which is capable of crossing the vascular endothelial barrier. Consistent with this idea, the percentage of IGF-I was significantly reduced in the 150-kDa ternary complex, with a concomitant increase in the percentage of free IGF-I in pregnancy plasma (25). In contrast to these findings, Suikkari and Baxter (26) have shown that the proteolyzed IGFBP-3 from pregnancy serum

can bind to IGFs and form a ternary complex with ALS of normal affinity. Thus, the functional significance of pregnancy-associated serum proteolysis of IGFBP-3 has not been fully established.

IGFBPs as potential modulators of local IGF actions

In addition to modulating the endocrine actions of IGF as described above, IGFBPs may have other roles.

To increase IGF bioavailability in specific tissues. Recent evidence suggests that IGFBP proteases may also regulate the bioavailability of IGFs in a tissue-specific manner. For example, it is known that the IGFBP-4-IGF complex is inactive, as this complex does not bind to IGF receptors, and that proteolysis of IGFBP-4 can increase the amount of local IGF available for IGF receptor interaction. Based on these findings and the observation that a number of local growth factors (*e.g.* TGFβ) can regulate IGFBP-4 proteolysis (23), it can be speculated that some effectors may increase the local production of IGFBP proteases, which, in turn, may degrade the inhibitory IGFBPs in extracellular fluid and thus increase the free level of IGFs for receptor interaction. Thus, tissue-specific regulation of IGFBP proteolysis may provide a mechanism to increase site-specific bioavailability of serum IGFs depending on local needs. In addition, the findings that IGFs regulate IGFBP levels by regulating proteolysis in a variety of cell types raise the interesting possibility that IGFs function to regulate IGFBPs, as well as *vice versa*. Future understanding of the identity, regulation, and roles of IGFBP proteases produced by various cell types will resolve these issues.

To facilitate storage of IGFs in extracellular matrexes. It is well known that growth factors, such as IGFs, are stored in relatively high abundance in certain extracellular matrexes, such as bone (27–29). Studies related to the mechanisms by which IGFs are stored in bone revealed that IGFBP-5, but not IGF-I or -II, can bind to hydroxyapatite and extracellular matrix proteins (27–29). These data suggest that IGFs are fixed in bone by means of IGFBP-5. The concept of a binding protein being involved in the storage of growth factors is not unique to IGFs, as TGFβ appears to be stored in bone via its binding protein. Although, the extent to which the IGFBPs in serum contribute to tissue fixation of IGFs is not clear, it is interesting to speculate that serum IGFBP-5 or locally produced IGFBP-5 may help to localize IGFs in a given tissue by binding to extracellular matrix proteins, thereby creating a growth factor depot in certain tissues for future actions. In this regard, we and others have proposed that growth factors fixed in bone may be released during bone resorption to stimulate nearby osteoblasts so as to ensure that there is a site-specific replacement of the resorbed bone. Similarly, IGFs stored in the extracellular matrexes of certain other tissues may promote wound healing and tissue regeneration.

To exert IGF-independent effects on target cells. The explosion of IGFBP research during the last few years has led to the novel concept that IGFBPs are not simply transport proteins, but, in addition, either inhibit or potentiate IGF actions (2). Recent studies on the mechanisms by which IGFBPs may mediate their effects on target cells have revealed an interesting possibility that some IGFBPs may have IGF-independent effects besides modulating the actions of IGF. For example, IGFBP-1 has been shown to stimulate smooth muscle cell migration in an IGF-independent manner involving binding to integrin receptor (30). IGFBP-3 and IGFBP-5 have also been shown to mediate their effects on breast cancer cells and bone cells, respectively, by IGF-independent mechanisms, possibly involving IGFBP-specific cell surface binding sites (31–33). Valentinis *et al.* (34) have shown that overexpression of IGFBP-3 in fibroblasts derived from IGF-I receptor knockout mouse embryo inhibited cell growth, suggesting that the inhibitory effect of IGFBP-3 does not involve signal transduction via type I IGF receptor. Recent studies have also provided evidence for the presence of putative IGFBP-binding sites on the cell surface (31–33). These data support the unique possibility that serum IGFBPs may modulate their effects on target tissues depending on local needs by not only regulating the endocrine actions of IGF, but also by having IGF-independent direct effects on target cells. However, further studies involving the identification of signal-transducing IGFBP receptors and establishment of a cause and effect relationship between IGFBP binding to its potential receptor and IGFBP actions are needed to verify that IGFBPs may in part mediate their effects independent of IGFs. This is clearly an exciting area for future investigations.

In conclusion, all important regulatory systems in biology are complex, and the IGF regulatory system is no exception. The last few years have brought complexity, but also new vistas of insights into the IGF system with the discovery of new IGFBPs and new putative IGFBP proteases. One can envision very exciting times in the future as the scientific community seeks to advance the frontiers of both the molecular mechanisms of the IGF system and also the bigger picture of the function of the IGF system as a hormone and a local signaling system.

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