REVIEW

The effect of GH and IGF1 on linear growth and skeletal development and their modulation by SOCS proteins

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Abstract

Circulating signalling proteins have often been divided into hormones and cytokines, but it is increasingly being recognised that these substances have a number of common characteristics and mechanisms of action. This is clearly illustrated by the suppressor of cytokine signalling (SOCS) proteins which are increasingly seen as a central component of the regulation of the action of hormones and cytokines that signal through the cytokine receptor complex. The SOCS protein family is probably more extensive than currently recognised; its members may have differential tissue expression and their potency for suppressing cytokine signalling may vary. Recent knockout and transgenic studies in mice have highlighted the role that these proteins play in growth and skeletal development as well as in inflammation. Chronic inflammation is associated with altered growth and skeletal development, and it is possible that SOCS proteins may have an important role to play in mediating these effects.
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Introduction

Linear growth and skeletal development are tightly regulated processses that are highly dependent on GH signalling and action. Clinical studies have shown that growth and skeletal development are impaired during periods of uncontrolled chronic inflammation which is often associated with altered systemic and local cytokine milieu. The mechanisms by which these inflammatory cytokines modulate linear growth and skeletal development are poorly understood, but an involvement of members of the suppressor of cytokine signalling (SOCS) family has been proposed. This review will first describe SOCS proteins and the effects of the GH/insulin-like growth factor 1 (IGF1) axis on linear growth and skeletal development before describing the evidence that highlights the role of SOCS proteins in controlling this axis as well as in skeletal development.

Cytokine signalling

Cellular responses to cytokine stimulation depend on the type of cytokine and the nature of the target cell, and include immune function, inflammation, and cell proliferation and differentiation. The interaction between a cytokine and its receptor induces receptor dimerisation or oligomerisation, which results in the juxtaposition of a group of proteins that are members of the Janus kinase (JAK) family of protein tyrosine kinases – JAK1, JAK2, JAK3 and TYK2 (Ihle & Kerr 1995). These can cross-phosphorylate, causing enzymatic activation of the cytokine receptor. GH, prolactin and leptin are just some of the ligands that signal through these same receptors and along with other cytokines have their own specific preferential JAKs. A key target of JAK activity is the cytoplasmic domain of the cytokine receptor, which becomes tyrosine phosphorylated at multiple residues, creating docking sites for signalling proteins containing Src homology 2 (SH2) or phosphotyrosine-binding domains. It is likely that some downstream signalling pathways are common to all cytokine receptors and some are specific for individual cytokine receptors (Leaman *et al*. 1996). The association between signalling proteins and cytokine receptors serves to initiate multiple signalling pathways, such as those regulated by Ras sarcoma proteins (RAS), phosphatidylinositol 3-kinase (PI3K) and the signal transducers and activators of transcription (STATs). Together, these pathways culminate in the regulation of gene expression in the nucleus, resulting in an appropriate cellular response to the cytokine. The STAT family, consisting of at least seven transcription factors, plays a critical role in regulating physiological responses to cytokine stimulation. Members of

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the STAT family bind tyrosine-phosphorylated cytokine receptors through their SH2 domains. Once bound to the receptor, STATs are phosphorylated by JAKs, following which they dissociate from the receptor and form homo- or heterodimers. STAT dimers then translocate to the nucleus, where they interact with specific DNA elements in the promoters of cytokine-responsive target genes and thus regulate transcription (Darnell 1997). The transcriptional activity of STATs may depend on a number of factors including its interaction with other proteins within the cell. For instance, STAT5 transcriptional activity is increased by formation of a complex with the glucocorticoid or mineralocorticoid receptor, while it is reduced by formation of a complex with the oestrogen receptor (Stoecklin *et al.* 1999).

SOCS proteins

Suppression of signalling through the activated cytokine receptor can occur by receptor degradation through the ubiquitin/proteosome pathway or by dephosphorylation of tyrosines within JAK or the receptor. The SOCS proteins are an important group of proteins that are generated in response to cytokines and can also bind through their SH2 domains to phosphorylated tyrosines within the cytokine receptor–JAK complex, and inhibit further cytokine receptor activation (Hilton 1999). These proteins may also promote proteosomal degradation of the JAKs (Zhang *et al.* 1999). The SOCS family contains at least eight members: SOCS1–7 and the cytokine inducible SH2-containing protein (CIS). Although SOCS1, 2, 3 and CIS are well characterised, little is known about the function and mechanisms of action of SOCS4–7. Structurally, SOCS proteins possess a poorly conserved amino-terminal domain of variable length, a central SH2 domain, as well as a highly conserved amino acid C-terminal domain, named the SOCS box (Fig. 1). Although the basal levels of SOCS proteins are generally low, their expression levels have been shown to be markedly induced by numerous cytokines, growth factors and hormones, such as interleukin 1 (IL1), IL2, IL3, IL4, IL6, IL9, IL10, IL11, interferon α (IFN-α), IFN-γ, insulin, ciliary neurotrophic factor, granulocyte colony-stimulating factor, leukaemia inhibitory factor, GH, angiotensin II, cardiotrophin, oestrogen, prolactin and thyrotrophin. The transcriptional regulation of SOCS proteins appears to be mediated, at least in part, by the STAT signalling pathway. Indeed, the promoter region of *SOCS* genes, such as the *murine Socs3*, may have specific sequences for STAT binding (Auernhammer *et al.* 1999) or for other hormones such as oestrogen (Leong *et al.* 2004). Accordingly, cells transfected with a dominant negative mutant of STAT3 failed to induce SOCS expression following IL6 stimulation. Furthermore, SOCS proteins, particularly, murine SOCS2, 6 and 7, may be able to regulate the degradation of other members of their family (Piessevaux *et al.* 2006). Finally, the SOCS family of proteins may be larger than the eight that have been hitherto described. There are up to 20 proteins with similar SOCS motifs, and there are also other proteins such as caveolin 1, which can also suppress cytokine signalling by inhibiting the kinase activity of JAK family members (Jasmin *et al.* 2006).

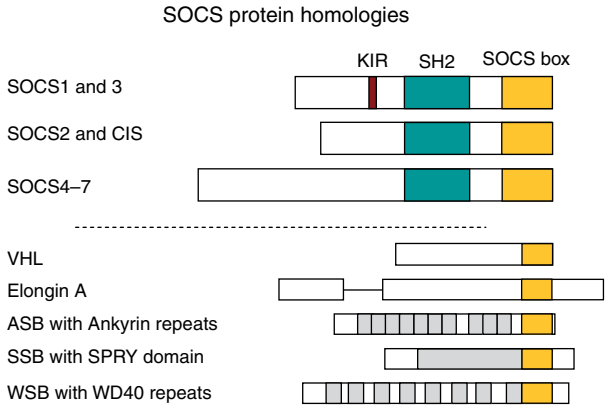

Figure 1 Diagram representing the structure of SOCS proteins. Eight proteins that belong to the SOCS family of proteins are shown in the upper panel. They are characterised by the presence of an Src homology 2 (SH2) central domain and the SOCS box domain at the C-terminus. A small domain called kinase inhibitory region (KIR), only found in SOCS1 and SOCS3, is shown as a small box at the N-terminal region. SOCS proteins can interact with phosphorylated tyrosines through their SH2 domain and with Elongin BC through their SOCS box domain. Some other proteins containing a SOCS box domain but lacking a SH2 domain are shown in the lower panel.

The GH/IGF1 axis

GH and IGF1 are important regulators of longitudinal growth. GH is a single-chain peptide of 191 amino acids. The synthesis and release of GH from the anterior pituitary gland are promoted by GHRH, and inhibited by somatostatin but regulated by a range of central and peripheral signals (Goldenberg & Barkan 2007). IGF1, which is secreted by the liver under GH control, inhibits GH secretion directly in somatotrophs and indirectly by stimulating the release of somatostatin (Goldenberg & Barkan 2007). GH circulates bind to a GH-binding protein, which is the extracellular domain of the GH receptor (GHR; Bougnères & Goffin 2007). The function of the GH-binding protein is incompletely understood, although it may modulate the activity of GH either by prolonging its half-life or by reducing its availability to the GHR.

In the human, the GHR is highly expressed in the liver, adipose tissue, heart, kidneys, intestine, lung, pancreas, cartilage and skeletal muscle where it induces the synthesis of IGF1 (Ballesteros *et al.* 2000). However, systemic IGF1 which is synthesised primarily in the liver circulates as part of a 150-kDa complex formed by one molecule each of IGF1, IGF-binding protein (IGFBP)-3, the predominant circulating-binding protein, or IGFBP-5, and the acid labile subunit

(ALS). There are six IGFBPs, and IGFBP-1, -2, -4 and -6 also can bind IGF1 in the circulation and peripheral tissues but do not form part of the ternary complex (Holly & Perks 2006). IGFBPs are in concentrations in excess of IGF1. Consequently, IGF1 circulates mostly bind to the complex, and <1% of total serum IGF1 circulates as a free hormone. The 150-kDa ternary complex stabilises IGF1, prolonging its circulating half-life and regulating its availability to target tissues. Consequently, the ternary complex plays an important role in determining the endocrine function of IGF1. Although, in excess, IGFBPs inhibit IGF1 action, the triple inactivation of IGFBP-3, -4 and -5 demonstrated that IGFBPs are necessary to maintain appropriate levels of systemic IGF1 and adequate postnatal growth (Ning *et al.* 2006). ALS is synthesised in the liver under the control of GH and circulates in excess over the other components of the complex, so that it plays a critical role in the storage and release of IGF1. IGF2 shares biochemical and biological properties with IGF1; it is important in skeletal development, but its function in the adult skeleton is not proven. IGF2 is synthesised by skeletal cells, but its synthesis is not GH dependent. The IGF2/mannose-6-phosphate receptor does not play a major role in IGF signal transduction and is responsible for clearing IGF2, regulating its levels, during foetal development (Blackburn *et al.* 1997).

The GH/IGF1 axis and bone

Bone is a dynamic connective tissue that undergoes a continuous process of resorption and renewal. The principal cells that mediate this process include the osteoprogenitor cells that contribute to maintaining the osteoblast population, the osteoblasts that sythesise the bone matrix, the osteocytes that influence bone structure and response to mechanical load and the osteoclasts that promote bone resorption.

Osteoblast maturation and function requires a spectrum of signalling proteins including morphogens, hormones, growth factors, cytokines, matrix proteins and transcription factors that act in a temporal-specific manner (Aubin *et al.* 2006). Through the PI3K pathway, IGF1 may reduce osteoblast apoptosis and promote osteoblastogenesis by stabilising β-catenin, enhancing Wnt-dependent activity (Playford *et al.* 2000, Krishnan *et al.* 2006). This effect, associated with modest mitogenic properties, causes an increase in the number of osteoblasts, and an increase in osteoblastic function and bone formation (Canalis 1980). The effect of IGF1 on bone resorption is less clear than on bone formation. IGF1 induces RANK-L synthesis (and as a consequence osteoclastogenesis) and enhances osteoclast function (Mochizuki *et al.* 1992). IGF1 induces vascular endothelial growth factor (VEGF) expression in skeletal cells, and VEGF may serve to couple angiogenesis with endochondral bone formation and with osteoblastic differentiation and function (Akeno *et al.* 2002). Transgenic mice expressing IGF1 under the control of the osteoblast-specific osteocalcin promoter exhibit increases in trabecular bone secondary to an increase in bone formation (Zhao *et al.* 2000). *Igf1* null mutants exhibit reduced cortical bone but not trabecular bone, possibly due to a compensatory increase in GH secretion or due to a decrease in trabecular bone resorption (Liu *et al.* 1993). Mice carrying mutations of the GHRH receptor (lit/lit mouse) or the *GHR* have no GH secretion or action, and consequently low levels of systemic IGF1 (Beamer & Eicher 1976, Sims *et al.* 2000). These mutants display osteopenia and reduced cortical bone, but display normal trabecular bone. The contribution of systemic IGF1 to cortical bone integrity is confirmed in mice carrying a liver-specific *igf1* deletion singly or in combination with an *als* deletion. These mice, which display reductions in serum IGF1, have decreased cortical bone (Yakar *et al.* 2009). These observations confirm the contribution of systemic IGF1 to cortical bone integrity and to a lesser extent to trabecular bone integrity. In contrast, the locally produced skeletal IGF1 plays a more significant role in trabecular bone integrity. This is demonstrated in transgenic mice expressing IGF1 in osteoblasts and in conditional *igf1* receptor null mice, which display decreased osteoblast number and function, causing reduced bone formation and reduced trabecular bone volume (Zhao *et al.* 2000, Zhang *et al.* 2002). Therefore, systemic IGF1 maintains cortical bone structure, whereas skeletal IGF1 serves to maintain trabecular bone structure. The function of IGF1 in skeletal homeostasis is confirmed in *irs1* or *irs2* null mutants, which also exhibit osteopenia (Gazzerro & Canalis 2006). At a local level, although GH is not a major inducer of IGF1 in osteoblasts, parathyroid hormone (PTH) and other inducers of cAMP increase IGF1 expression in osteoblasts, and IGF1 may, therefore, mediate some of the actions of PTH in bone *in vitro* and *in vivo* (Canalis *et al.* 1989). On the other hand, glucocorticoids decrease IGF1 transcription in osteoblasts, and their inhibitory effects on the function of the mature osteoblast may be partly explained by reduced IGF1 levels in the bone microenvironment (Delany *et al.* 2001).

For osteoclastogenesis, two cytokines, RANKL and MCSF play a very important role. The discovery of RANKL, a member of the tumor necrosis factor (TNF) superfamily, was preceded by the identification of its naturally occurring inhibitor, osteoprotegerin (OPG) which is produced by osteoblasts. Optimal osteoclast function depends on the creation of a suitable microenvironment that facilitates bone resorption, and this requires an acidic milieu, the presence of a lysosomal enzyme cathepsin K and the presence of integrins that facilitate the physical intimacy between the osteoclast and the bone matrix. In osteoclastogenesis, growth factors, such as transforming growth factor β (TGF-β), which is the most abundant cytokine in the bone matrix, play an important role in maintaining and enhancing the responsiveness of osteoclast precursors to RANKL (Fuller *et al.* 2000). Osteoclasts are reported to express GH and IGF1 receptors in the mammal (Zhang *et al.* 1992, Fiorelli *et al.* 1996, Hou *et al.* 1997), and *in vivo* or *in vitro* exposure to recombinant GH is associated with an increase in markers of bone resorption (Kassem *et al.* 1994) and increased osteoclast activity (Guicheux *et al.* 1998).

GH and IGFBP-5 exposure can increase osteoclast activation and can stimulate osteoclastogenesis; it is possible that the former effect may be dependent on the presence of osteoblasts, but promotion of osteoclast differentiation from haematopoietic blast cells seems to be an osteoblast-independent effect (Nishiyama *et al.* 1996, Kanatani *et al.* 2000). Furthermore, the GH induced-osteoclast differentiation seems to be independent of IGF1, whereas the activation of osteoclast is dependent on IGF1 (Kanatani *et al.* 2000). Thus, unlike bone formation, where the stimulatory effects of GH seem to be via IGF1, GH seems to have a more profound independent stimulatory effect on bone resorption. Pro-inflammatory cytokines such as TNF-α, IL1β and IL6 can promote osteoclastogenesis, and GH and IGF1 can stimulate production of these cytokines in osteoblasts (Slootweg *et al.* 1992, Swolin & Ohlsson 1996) and in T-cells (Renier *et al.* 1996, Uronen-hansson *et al.* 2003). *In vivo* and *in vitro* studies suggest that GH and IGF1 may also influence osteoclast activity by altering the RANKL/OPG balance, but there is a lack of clarity about the direction and magnitude of this effect (Ueland 2005).

The GH/IGF1 axis and the growth plate

The original somatomedin hypothesis proposes that GH stimulates growth at the epiphysis by systemically derived liver IGF1 (Salmon & Daughday 1957, Daughaday *et al.* 1972). The somatomedin hypothesis has been questioned as direct effects of GH on chondrocytes *in vivo* and *in vitro* have been reported (Isaksson *et al.* 1982, Madsen *et al.* 1983, Isgaard *et al.* 1986, Schlechter *et al.* 1986). Although such direct effects have not been observed by others (Burch *et al.* 1985, Makower *et al.* 1989), an alternative dual effector theory of GH action has been proposed (Green *et al.* 1985). This involves GH acting on germinal zone precursors of the growth plate to stimulate the differentiation of chondrocytes and then amplify local IGF1 synthesis, which, in turn, induces the clonal expansion of chondrocyte columns and hypertrophy in an autocrine/paracrine manner (Isaksson *et al.* 1982, Green *et al.* 1985, Zezulak & Green 1986). While concentration of the GHR within the germinal chondrocytes of the growth plate is consistent with the dual effector theory (Barnard *et al.* 1988), recent *in situ* hybridisation studies and immunohistochemical investigations using more specific GHR antibodies have indicated a broader distribution of the GHR within the growth plate suggesting additional roles for GH such as the regulation of chondrocyte proliferation, differentiation and hypertrophy (Lupu *et al.* 2001, Gevers *et al.* 2002). Interestingly, this study by Gevers *et al.* also indicated that the chondrocytes of the growth plate expressed GH-binding protein where it may prolong the half-life of GH *in vivo* or alternatively it may compete with the GHR for binding to GH and protect chondrocytes from continuous GH exposure. In addition to GH, IGF1 has also been shown to stimulate the proliferation of germinal zone chondrocytes, and therefore a role for systemic or local IGF1 in initiating chondrocyte events in the germinal zone of the growth plate cannot be excluded (Hunziker *et al.* 1994, Reinecke *et al.* 2000).

Further studies using conditional liver *Igf1* knockout mice have also challenged the classical somatomedin hypothesis (Sjögren *et al.* 1999, Yakar *et al.* 1999). Both the studies reported a significant reduction in circulating IGF1 but not in bodyweights of the transgenic mice suggesting that while liver-derived IGF1 is the main determinant of circulating IGF1 levels, it is not as important for postnatal growth as locally derived IGF1. These observations have, however, been questioned by others (Lupu *et al.* 2001, Stratikopoulos *et al.* 2008) who have suggested that the endocrine ablation of liver IGF1 only occurred after the critical postweaning growth spurt. Also liver IGF1 production was achieved in mice lacking *Igf1* gene expression in all other tissues demonstrating that under these conditions, endocrine IGF1 plays a very significant role in mouse growth as its action contributes 30% of adult body size and sustains postnatal development (Stratikopoulos *et al.* 2008).

The relative contributions of GH and IGF1 to pre- and postnatal bone growth have been examined through growth analysis of various transgenic mouse lines. GH deficiency results in impaired growth, and the growth of *ghr* null mice is retarded from ~2 weeks after birth (Lupu *et al.* 2001). In contrast, IGF1 deficiency retards both pre- and postnatal growth, and *igf1r* null mice, while exhibiting a more severe growth deficiency, die shortly after birth (Baker *et al.* 1993, Liu *et al.* 1993, Lupu *et al.* 2001). In this context, prenatally, IGF1 signalling is considered to be GH independent, whereas postnatally, IGF1 is partly or fully GH dependent with phosphorylation of STAT5b having an intermediary role (Herrington *et al.* 2000, Woelfle *et al.* 2003, Klammt *et al.* 2008). Under specific conditions, both GH and IGF1 can promote skeletal growth. Mice overexpressing GH have increased growth, but this is only observed at 3 weeks postnatally, despite high circulating GH levels at birth. This accelerated growth coincides with a delayed induction of IGF1 expression strongly suggesting that IGF1 is directly involved in mediating the GH signal (Mathews *et al.* 1988*a*). Chronic overexpression of IGF1 does not, however, accelerate skeletal growth, whereas *igf1* transgenic GH-deficient mice display normal linear growth (Mathews *et al.* 1988*b*, Behringer *et al.* 1990). This latter observation (Behringer *et al.* 1990) suggests that IGF1 can mediate GH function in regulating growth. Also, mutant mice lacking IGF1 exhibit increased growth after IGF1 administration but are unresponsive to GH which may indicate that GH itself makes a smaller contribution to growth (Won & Powell-Braxton 1998, Lupu *et al.* 2001). However, if all growth-promoting GH actions are mediated by IGF1, the expectation would be that the phenotype of *ghr* and *igf1* null mice would be indistinguishable from double *ghr/igf1r* mutants. This has been shown not to be the case. Both *ghr* and *igf1* null mice show reduced tibial growth that is more severe in double *ghr/igf1r* mutants, and it is likely therefore that GH and IGF1 have both independent and common functions (Lupu *et al.* 2001, Wu *et al.* 2009). The independent functions of GH and IGF1 on the chondrocytes of the growth plate may, however, predominate. In comparison with the control mice, the growth deficits of the

tibia in the double *ghr/igf1r* null mice are almost identical to the sum of growth deficit observed in the single *ghr* and *igf1* mutant tibia. The contributing overlapping function has been estimated not to exceed 5% (Lupu *et al.* 2001). All contributions considering the growth-promoting role of GH are well accepted, but the relative contributions to the growth of the direct or indirect effects of GH have still to be precisely determined.

The actions of IGF1, whether produced locally or systemically, are via the IGF1 receptor (IGF1R) expressed on the cell surface of the chondrocytes of the growth plate. The type-2 IGF1R is expressed equally throughout all maturational zones of the growth plate, whereas the type-1 receptor is more highly expressed by proliferating chondrocytes (Trippel *et al.* 1986, Parker *et al.* 2007). These data are consistent with the concept that IGF1 has regulatory actions on all chondrocytes of the growth plate. The IGF1 signalling pathway has a central function in modulating endochondral bone growth and regulates a number of key chondrocyte physiological processes such as chondrocyte proliferation, matrix synthesis, differentiation, hypertrophy and survival (Lupu *et al.* 2001, van der Eerden *et al.* 2003, Wang *et al.* 2004, Nilsson *et al.* 2005). Some dispute, however, exists concerning the major physiological drivers on IGF1-enhanced bone growth. Chondrocyte numbers and proliferation rates are reported to be normal (Wang *et al.* 1999) or decreased (Lupu *et al.* 2001) in *igf1* null mice, whereas the size of the hypertrophic chondrocytes are smaller in the *igf1* mutant mice (Wang *et al.* 1999). The direct effects of IGF1 on bone growth have been investigated in cultured rodent metatarsals maintained in culture. Under these highly controlled conditions, IGF1 increased chondrocyte proliferation, hypertrophic cell size and linear growth (Scheven and Hamilton 1991, Mushtaq *et al.* 2004). One important outstanding question yet to be fully clarified is the cellular source of the IGF1 (GH dependent or independent) that controls linear bone growth. Contrasting data exist on the presence (Nilsson *et al.* 1990, Reinecke *et al.* 2000) or absence (Shinar *et al.* 1993, Wang *et al.* 1995) of *IGF1* mRNA in the chondrocytes of the growth plate which may result from limitations of the methodologies employed. Recently, a combination of growth plate microdissection and quantitative PCR has revealed that *IGF1* mRNA levels are very low in rat chondrocytes of the growth plate suggesting that the biological importance of this source of IGF1 may be negligible (Parker *et al.* 2007). Interestingly, these authors suggest that the source of IGF1 interacting with its chondrocyte receptor may be derived from the plasma or surrounding perichondrium and/or bone (Parker *et al.* 2007).

The SOCS proteins and their effect on growth

It is well recognised that GH/IGF1 signalling is modulated by CIS and SOCS1–3, but surprisingly, apart from studies reporting the role of SOCS2 in bone growth, little information exists on the effects of the other SOCS protein in regulating linear growth. Recent genome wide association studies have identified SOCS2 as one of 20 loci that influence human adult height (Weedon *et al.* 2008) This section of the review is limited to relevant data on SOCS1–3. The effect of these SOCS proteins on GH signalling is summarised in Fig. 2.

SOCS1

Analysis of *socs1* null mice has shown that this protein is indispensable for normal postnatal development. At birth, *socs1*-deficient mice are indistinguishable from their normal littermates, but within 10 days the *socs1* null mice exhibit stunted growth and die within the first 3 weeks of life. The smaller body weight of *socs1*-deficient mice was associated with an abnormal femoral marrow cell count, and cytological analysis revealed a consistent deficit of lymphocytes in this population (Starr *et al.* 1998). The observed growth retardation is likely to be a pathological response to uncontrolled IFN-γ signalling observed in *socs1* null mice as treatment of *socs1* null mice with neutralising anti-IFN-γ antibody resulted in a healthy phenotype and no growth retardation at 3 weeks of age (Alexander *et al.* 1999). Almost identical data were obtained in double *socs1*$^{-/-}$/*ifn-γ*$^{-/-}$

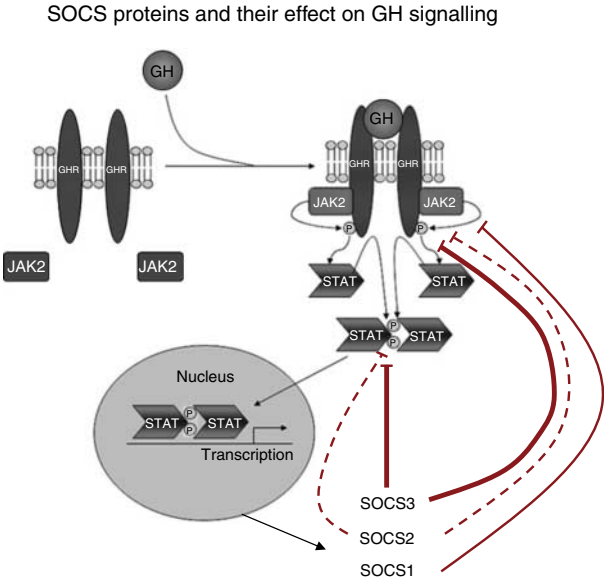

Figure 2 Inhibition of GH signal transduction by SOCS molecules. SOCS1, SOCS2 and SOCS3 can each inhibit signalling downstream of GHR activation; however, they do so by different mechanisms. SOCS1 binds to JAK and inhibits its ability to phosphorylate the receptor, whereas SOCS3 and SOCS2 bind to phosphorylated tyrosines that might also be STAT5-binding sites, such that SOCS2 might block STAT5 binding and thus inhibit the phosphorylation, dimerisation and transcriptional activation of STAT5. In addition, the SOCS proteins may promote proteasomal degradation of their targets. SOCS proteins, particularly, SOCS2 and SOCS3 have different levels of potency, and the final biological effect may depend on the relative concentration of the two proteins. The strength of the red lines reflects the respective potency of the inhibitory effect of SOCS1–3. Adapted from Pass *et al.* (2009).

mice where the authors reported no pathology and normal body growth at 3 weeks of age (Alexander *et al.* 1999). It is unreported whether *socs1* null mice have elevated GH signalling, but this would be expected as inhibition of GH signalling by SOCS1 is complete (Adams *et al.* 1998, Hansen *et al.* 1999). Although an increased growth phenotype as observed in the *socs2* null mice (see below) would be expected in the *socs1* null mice, this has not been reported in *socs1*$^{-/-}$/*ifn-γ*$^{-/-}$ mice maintained for up to 6 months of age (Alexander *et al.* 1999).

SOCS2

The phenotype of *socs2* null mice is notable due to its increase in linear bone growth and body mass where there is a proportionate augmentation of most visceral organs (Metcalf *et al.* 2000, Macrae *et al.* 2009). SOCS2 deficiency results in a 40% increase in body weight of 6-week-old male mice and a 27% increase in the body weight of 7-week-old female mice (MacRae *et al.* 2009). Body length in male and female *socs2*-deficient mice is also greater as is the length of the long bones of the fore and hind limbs (Metcalf *et al.* 2000, Lorentzon *et al.* 2005, Macrae *et al.* 2009). The increased longitudinal bone growth observed in *socs2* null mice (Metcalf *et al.* 2000, Lorentzon *et al.* 2005, MacRae *et al.* 2009) is consistent with increased signalling through the GH/IGF1 axis and indicates that SOCS2 protein has a functional role in the chondrocytes of the growth plate dynamics. Nevertheless, initial histological analyses failed to record any obvious abnormalities of the epiphyseal growth plate of the tibia and femur (Metcalf *et al.* 2000). A fuller histomorphometric analysis, however, revealed that *socs2* null mice had wider growth plates with significantly wider proliferative and hypertrophic zones (MacRae *et al.* 2009). SOCS2 gene and protein are expressed preferentially by proliferating the chondrocytes of the growth plate suggesting that the increased bone growth and observed structural differences within the growth plate observed in *socs2* null mice are direct consequences of altered SOCS2 (MacRae *et al.* 2009). Also, based on observations on other cell types, it is possible that SOCS2 may be linked to the transition from the proliferative to the differentiated chondrocyte phenotype (Goldshmit *et al.* 2004, Wang *et al.* 2004, Ouyang *et al.* 2006). The effect of SOCS2 deficiency on chondrocyte proliferation, apoptosis and matrix synthesis has yet to be determined.

Enhanced growth of *socs2* null mice is not observed until 3–4 weeks of age (Metcalf *et al.* 2000, Macrae *et al.* 2009), and this is consistent with the concept that SOCS2 interacts with GH to negatively regulate GH function. Inhibition of growth in GH-deficient mice is not observed until ~14 days after birth, and peak GH activity occurs between postnatal days 20 and 40 (Lupu *et al.* 2001, Wang *et al.* 2004). Additionally, the increased growth of *socs2* null mice is not observed when mutant mice are mated with either mice lacking the *STAT5b* gene or those with a point mutation in the Ghrhr lit/lit mice (Greenhalgh *et al.* 2002, 2005). Furthermore, administration of GH to *socs2*$^{-/-}$/*Ghrhr lit/lit* mice caused an increase of growth to a size indistinguishable from *socs2* null mice (Greenhalgh *et al.* 2005). These observations provide further proof that the *socs2*$^{-/-}$ overgrowth phenotype is dependent on aberrant GH signalling. While the role of SOCS2 in down-regulating GH signalling is widely accepted, its role in inhibiting IGF1 action is more speculative, although SOCS2 has been shown to bind to the IGF1 receptor and limit the growth-promoting actions of IGF1 *in vivo* (Dey *et al.* 1998, Michaylira *et al.* 2006). Interestingly, most organs in *socs2* null mice are enlarged, including those in which elevated IGF1 is not detected which possibly indicates that SOCS2 is also required for the regulation of IGF1 signalling itself (Metcalf *et al.* 2000). Indeed, a role for SOCS2 in regulating both GH and IGF1 signalling in organ-specific contexts is consistent with the observation that *socs2* null mice exhibit characteristics of both GH and IGF1 transgenic mice without entirely recapitulating either phenotype (Metcalf *et al.* 2000).

In vitro data have further discussed the biological role of SOCS2 in mediating GH action. At low concentrations, SOCS2 inhibits GH signalling (Ram & Waxman 1999). However, higher concentrations of SOCS2 restore and even stimulate GH signalling, suggesting a further positive modulatory role for SOCS2 in restoring the sensitivity of inhibited GH signalling in circumstances when it is suppressed by other SOCS proteins (Favre *et al.* 1999, Greenhalgh *et al.* 2002). *In vivo* studies in mice demonstrating that both the absence and overexpression of SOCS2 cause growth enhancement support these *in vitro* findings (Metcalf *et al.* 2000, Greenhalgh *et al.* 2002). It has, therefore, been proposed that SOCS2 regulates growth by exerting a dual effect on GH signalling. Both inactivation and overexpression of SOCS2 result in enhanced GH signalling and growth, whereas physiological levels of SOCS2 reduce GH signalling. Glucocorticoids, including dexamethasone, are also thought to upregulate SOCS2, desensitise GH signalling and suppress growth (Tollet-Egnell *et al.* 1999, Rico-Bautista *et al.* 2006).

A similar phenotype to *socs2* null mice has also been observed in the high growth (hg) mouse, a phenotype that occurs following a spontaneous 500-kb deletion in chromosome 10 of the mouse (Horvat & Medrano 1995, 1998, 2001). Since the initial discovery of the hg phenotype, the *SOCS2* gene has been mapped to the hg chromosomal region possibly explaining the almost identical phenotype: a 30 to 50% increase in postnatal growth (Horvat & Medrano 2001). Interestingly, however, unlike the *socs2* null mice, the *hg* mice have elevated levels of circulating IGF1 which may be due to a deletion of other genes or DNA segments flanking the SOCS2 locus (Horvat & Medrano 2001).

SOCS3

Mice lacking SOCS3 expression are embryonically lethal with death occurring at mid-gestation between days 11 and 13 of embryonic development. At this stage, the embryos appear normal but present with a mild growth retardation. The developmental arrest and death were considered to be due to defects in placental development possibly as a result of excess cytokine signalling (Marine *et al.* 1999, Roberts *et al.* 2001).

In comparison to *CIS*, *SOCS1* and *SOCS2*, preferential induction of *SOCS3* mRNA by GH is observed in fibroblasts and hepatocytes. Also constitutive expression of SOCS3 (and SOCS1), but not SOCS2 or CIS resulted in a complete block of GHR-mediated signalling (Adams *et al.* 1998, Hansen *et al.* 1999). These data suggest, albeit not in the chondrocytes of the growth plate, that SOCS3 is preferentially stimulated by GH which results in the complete inhibition of GH signalling. The implications for this on bone growth are unknown due to the embryonic lethality of the *socs3* null mouse. Knowledge gleaned from studies with articular chondrocytes does, however, suggest that SOCS3 is likely to influence GH/IGF1 signalling in epiphyseal chondrocytes. SOCS3 overexpression of murine articular chondrocytes inhibits IL1-induced STAT1 and STAT3 phosphorylation as well as inhibits IGF1-induced aggrecan expression through antagonising insulin receptor substrate 1 phosphorylation (Smeets *et al.* 2006).

The SOCS proteins and their effect on bone

Given that the majority of studies performed have investigated the effects of SOCS1–3, this section has been restricted to the effect of these proteins.

SOCS1

Mice with homozygous inactivation of the gene encoding the SOCS1 protein die within 21 days of birth with low body weight, fatty degeneration and necrosis of the liver, infiltration of the lung, pancreas, heart and skin by macrophages and granulocytes and a profound depletion of T- and B-lymphocytes (Starr *et al.* 1998, Metcalf *et al.* 1999). In the *socs1* null mice, calvarial cells showed distinct phosphorylation of STAT1, but this was hardly detectable in wild-type (WT) mice (Abe *et al.* 2006). Undercalcified areas in the skulls and sternum, as well as comparatively thinner calcified areas in cortical bone, were found in *socs1* null mice. Mineralisation activity of primary cultured calvarial cells strongly suggested significant impairment in osteoblasts of *socs1* null mice. *In situ* hybridisation analysis demonstrated that these mice showed a dramatic decrease in the expression level of osteocalcin, a late marker of osteoblast maturation. Osteoclastogenesis stimulation by RANKL is associated with increased expression of IFN-β which itself inhibits the differentiation of osteoclasts. However, RANKL simultaneously induces the expression of SOCS1, which can block the signalling of IFN-β, thus causing a decrease in IFN-dependent transcription factor complex (IFN-stimulated gene factor-3) formation (Hayashi *et al.* 2002). Thus, although the inhibitory cytokines such as type-I IFNs are produced in response to RANKL, the inhibition of osteoclastogenesis may be rescued by inducing the production of signalling suppressors such as SOCS, and this was further confirmed in experiments of SOCS1 overexpression in mouse bone marrow-derived monocytes which conferred resistance to the suppression of osteoclast differentiation by IFN (Ohishi *et al.* 2005). Consistent with this report, a notable suppression of osteoclast formation and bone destruction induced by lipopolysaccharides have also been reported in *socs1*$^{+/-}$ mice which have a haploinsufficiency of the *socs1* gene (Ohishi *et al.* 2005).

SOCS2

Dual energy X-ray absorptiometry analysis of the *socs2* null mouse demonstrated that the areal bone mineral density (aBMD) was reduced in the total tibia. Subregion analyses in the proximal metaphyseal region of the tibia, with a relatively high content of trabecular bone, indicated that the reduced aBMD was due, at least partly, to a reduced trabecular BMD (Lorentzon *et al.* 2005). Peripheral quantitative computed tomography analyses demonstrated that both the trabecular and cortical volumetric BMD were reduced. The cortical cross-sectional area and cortical thickness were reduced in 4-week-old mice but not in 15-week-old *socs2*$^{-/-}$ mice, suggesting that the main effect on aBMD was a result of reduced trabecular and cortical volumetric BMD, and to a lesser extent due to reduced size of the cortical bone. However, more detailed studies performed recently using micro-computed tomography showed that although cortical and trabecular BMD were similar in the *socs2* null mice and WT mice, the tibiae in 7-week-old *socs2* null mice tibiae were longer, broader and had increased total cross-sectional bone area, increased percent bone volume, trabecular number and trabecular thickness, with associated decreases in trabecular separation (Macrae *et al.* 2009). The structure model index, which quantifies the characteristic form of a 3D structure in terms of amounts of plates and rods composing the structure (Hildebrand & Rüegsegger 1997), was also significantly lower in the tibiae from the *socs2* null mice indicating that the trabeculae in *socs2* null mice appeared to be more 'plate-like' and more connected, which is consistent with greater 'strength'. Osteocalcin and TRAP5b, respective markers of bone formation and resorption, were also reported to be higher in the *socs2* null mice. Thus, while the overall BMD may not be significantly altered, a number of microarchitectural markers of bone strength were significantly raised in both the trabecular and cortical compartments of bone in the *socs2* null mouse which is known to have increased growth without systemically raised concentrations of IGF1 (Metcalf *et al.* 2000). In the C2C12 mesenchymal precursor cell line, stable transfection of SOCS2 potentiated bone morphogenic protein-induced transdifferentiation of C2C12 cells into osteoblast phenotypes (Ouyang *et al.* 2006). This effect was observed to be due to regulation of JunB protein synthesis and was independent of the GH signalling pathway (Ouyang *et al.* 2006). These data suggest that it is possible that GH dependent and independent pathways may both be responsible for the bone phenotype of the *socs2* null mouse. The bone phenotype of the *socs2* transgenic mice has not yet been described (Greenhalgh *et al.* 2002).

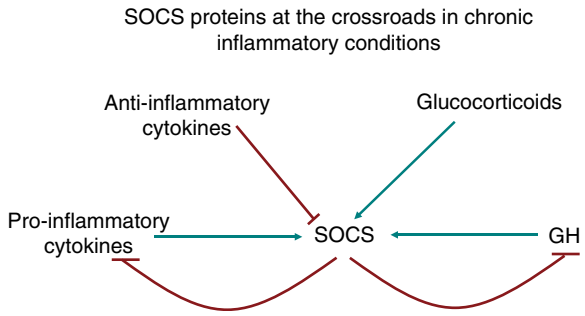

Figure 3 Hypothetical position of SOCS proteins as central modulators of the interaction between cytokines, glucocorticoids and GH. By altering the level of systemic or local SOCS proteins, these factors may induce an effect that alters the overall disease process.

SOCS3

The *socs3* null phenotype is embryonically lethal, and the effect of this genotype on bone is less clear. In osteoclastogenesis, SOCS3 seems to play a similar role as SOCS1 in dampening the inhibitory action of IFN-β on osteoclastogenesis (Hayashi *et al.* 2002, Lovibond *et al.* 2003). This inhibitory effect of SOCS3 on IFN-β may also be the pathway through which TGF-β facilitates osteoclast formation (Fox *et al.* 2003). In the osteoblast-like osteosarcoma cell line, UMR 106, which expresses a GH-responsive JAK2/STAT5 signalling system, pretreatment with 1,25 dihydroxy-vitamin D is associated with increased and sustained responsiveness to repeat pulses of GH stimulation. It seems that this may be due to a reduced GH-induced expression of SOCS3 and CIS (Morales *et al.* 2002).

Clinical and therapeutic relevance

Childhood inflammatory bowel diseases, especially those such as Crohn's disease, are commonly complicated by growth retardation and osteoporosis. Pro-inflammatory cytokines are often elevated in such conditions, and improvement of the disease status is often associated with an improvement in growth and skeletal health. Children with chronic inflammatory diseases show elevation of a range of anti- and pro-inflammatory cytokines (Wong *et al.* 2008). The systemic GH/IGF1 axis in these children can also show a range of abnormalities (Wong *et al.* 2010). GH therapy has been used to improve the growth of children with chronic diseases (Wong *et al.* 2007) and may alter systemic concentration of cytokines (Pagani *et al.* 2005, Andiran & Yordam 2007). The SOCS proteins may be central to the underlying pathophysiology of these observed effects (Walters & Griffiths 2009; Fig. 3) at systemic and local levels, and require further study.

Conclusion

In conclusion, effective GH signalling, particularly at the level of the target tissue, is vital for optimal linear growth and skeletal development. SOCS proteins are known inhibitors of GH signalling, but their actions vary from one family member to another and their role may differ depending on the target tissue. There is a need for more translational studies in this field to explore the role of SOCS proteins in mediating the effects of chronic inflammation on linear growth and skeletal development.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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