Cytokine actions in growth disorders associated with pediatric chronic inflammatory diseases (Review)

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Abstract. Growth disorders are commonly observed in children suffering from chronic inflammatory diseases such as Juvenile Idiopathic Arthritis (JIA) and Inflammatory Bowel Disease (IBD). These disorders range from general growth retardation to local acceleration of growth in the affected limb and are associated with the increased production of pro-inflammatory cytokines. In this article, we review how cytokines influence child growth by exerting a local effect at the level of the growth plate, and through systemic effects throughout the whole body.

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1. Introduction

Abnormal growth patterns are commonly observed in children suffering from chronic inflammatory diseases such as Juvenile Idiopathic Arthritis (JIA) and Inflammatory Bowel Disease (IBD), both at the onset of the disease and after steroid treatment. Changes in bone development are commonly associated with the growth abnormalities observed in these children. An imbalance of pro-inflammatory cytokines is

often seen in children with inflammatory diseases (1-5). The pro-inflammatory cytokines that have been reported to play a major role in JIA and IBD include interleukin-1ß (IL-1ß), tumour necrosis factor α (TNFα) and interleukin-6 (IL-6) (6-10).

Individual cytokines have multiple target cells and multiple actions, which often overlap with other cytokines. Cytokines may act as antagonists to or synergists with another, or may have a combined action altogether disparate to the individual agents. The action of cytokines is exerted via specific high-affinity cell surface receptors resulting in changes in gene expression of the target cell. Receptors of different cytokines share common signal transduction pathways allowing 'cross over' effects. Pro-inflammatory cytokines may act individually or in combination to influence child growth through systemic effects and/or a local effect at the level of the growth plate of long bones.

2. Growth and bone disorders associated with chronic inflammatory disease

In children suffering from JIA, estimates of significant short stature (final height SDS of <-2) range from 11% of patients with polyarticular JIA (11) to 41% of patients with systemic JIA (12). Child growth and skeletal development are reversibly impaired during periods of intensive glucocorticoid therapy (13). However, significant deviation of adult height from mid-parental height has been reported to be present only in children treated for longer than 12 months with systemic steroids (14). In a retrospective study of 24 children with systemic JIA (12), 87% of patients had a final height less than their target height. Furthermore, no catch-up growth was observed in 30% of the children following disease remission and the termination of glucocorticoid therapy. These patients were probably shorter at the time of diagnosis and had lower target heights (12).

Pubertal development is compromised in girls with JIA, particularly if they have received glucocorticoid therapy (15). Furthermore, girls with systemic JIA have a later menarche than those with polyarticular or oligoarticular JIA (15).

Children suffering from JIA show a distinctive pattern of growth disturbance; oligoarticular JIA is associated with localised excessive bone growth, whereas general growth retardation is often observed in children with systemic JIA. In the younger child with oligoarticular JIA, increased growth

in the affected limb occurs, with early fusion of the epiphyses leading to limb shortening in the older child (16). Early treatment of a young child with oligoarticular JIA with intra-articular glucocorticoids may avoid the leg length discrepancy, however this treatment may inhibit the growth of the contra-lateral leg (17,18).

Growth failure is a major feature of childhood IBD, and is more commonly observed in Crohn's disease (CD) rather than ulcerative colitis. Approximately 10-15% of children with CD have severe growth failure (height SDS <-2) at diagnosis (19,20). A prospective multi-centre study showed that approximately 21% of patients remain <-2 SDS after 2 years of follow-up (19).

Pubertal growth represents 15-20% of adult height, and precedes the fusion of the growth plates (21). Delayed puberty is common in patients with IBD, particularly CD (22,23). In a study of young patients with CD, menarche occurred at the age of 16 years or later in 73% of female patients in whom disease onset preceded puberty. In a few patients, menarche was delayed until the early 20's (23). In another study (24), the onset of puberty was taken as breast stage 2 in girls and testicular volume of 4 ml in boys. By these criteria, the mean age of onset of puberty was 12.6 years in young female patients with IBD compared with 11.1 years in healthy controls. In boys, the onset of puberty was 13.2 years in patients with IBD and 12.4 years in healthy controls (24). Furthermore, the duration of puberty may also be prolonged, particularly in patients with frequent disease relapses during this period (24).

Studies that have analysed the long-term growth of IBD patients from adolescence into adulthood have observed persistent stunting into adulthood at an incidence of 15-30% (25-27). A recent study of adult height in CD patients with pre-pubertal onset of symptoms showed that in the majority of patients (85%) the final height was less than 'target height', and in 22% the final height deficit was >10 cm (28).

Children suffering from JIA and IBD who show severe growth retardation may have normal pulsatile growth hormone (GH) secretion, but have reduced IGF-1 (insulin-like growth factor-1) levels suggestive of GH resistance (29). Two randomised controlled trials assessing the efficacy of recombinant human growth hormone (rhGH) in JIA have shown favourable results up to 4 years of treatment (30,31). A randomised placebo-controlled double blind cross over study using rhGH at 0.033 mg/kg/day showed significant improvement in height velocity and height SDS during treatment (30). A randomised controlled study using a moderately high rhGH dose of 0.05mg/kg/day showed that rhGH was effective in significantly improving height velocity SDS over a 4-year treatment period, and prevented the further deterioration in height SDS seen in those patients not treated with rhGH (31).

Only one prospective study has evaluated the response of rhGH in IBD patients (nine CD, one indeterminate colitis) with a history of chronic glucocortico-steroid exposure (32). rhGH administration at 0.05 mg/kg/day was associated with improved growth at 1 year from baseline and compared to controls. Improvement of height velocity from a mean of 3.5 cm/year at baseline to a mean of 7.7 cm/year was observed after 6 months of treatment. There was also a concomitant improvement of serum levels of IGF-1 and IGFBP-3 after treatment. This improvement was independent of disease activity and steroid dose (32). Preliminary results from a recent randomised controlled trial of GH in children with IBD showed improved growth in the rhGH-treated group at 1 year from baseline (33). However, pilot results from another randomised trial of rhGH at 0.05 mg/kg/day for children with CD (3 patients) versus placebo treatment (4 patients) showed no improvement in growth for the pooled result of growth for all 7 patients during rhGH treatment. The 4 patients treated with placebo were subsequently treated with rhGH (34).

Overall, these studies suggest that moderately high doses of rhGH can improve short-term height velocity, prevent further deterioration of linear growth and reverse the catabolic state induced by the inflammatory process and chronic steroid use.

A direct association between factors produced during chronic inflammation and growth failure has been proposed (29,35,36). In patients with systemic JIA, inhibition of linear growth is observed during disease activity periods, with subsequent growth rate normalisation during remission (35,36). In children with systemic JIA and growth retardation, growth velocity during GH treatment is inversely correlated with inflammation intensity (29). Pro-inflammatory cytokines may be associated with the growth failure observed in JIA and IBD by acting through both systemic effects and/or locally at the level of the growth plate.

3. Systemic and local control of bone growth

Articular chondrocytes produce and maintain an extracellular matrix (ECM) that is able to assist joint articulation and resist physical deformation. Growth plate cartilage is progressively synthesised at the epiphyseal growth plate and subsequently replaced by bone, with accompanying longitudinal bone growth (37).

The growth plate is a thin cartilage layer located near the ends of vertebrae and long bones (38). It consists of both chondrocytes and their ECM, where proteoglycans and collagen type II predominate. A feature of endochondral bone growth is the exact chronological and spatial organisation of chondrocytes in the growth plate. The chondrocytes differentiate through a sequence of maturational phases whilst staying in a fixed location (39) (Fig. 1).

Undifferentiated progenitors within the reserve stem cell zone differentiate into chondrocytes and advance through a proliferative phase. Immediately after the termination of cell division, the cells undergo terminal differentiation into hypertrophic chondrocytes (40). These chondrocytes are considerably larger, with increases in Golgi apparatus and rough endoplasmic reticulum, reflecting greater ECM synthesis (41). The rate of endochondral bone growth attributed to a specific growth plate in any given period of time results from a complex synchronisation of proliferative kinetics, ECM production, and hypertrophic chondrocyte enlargement (42). The exact control of these processes is yet to be elucidated, and any alteration of these variables by external factors such as pro-inflammatory cytokines may induce growth modulatory effects.
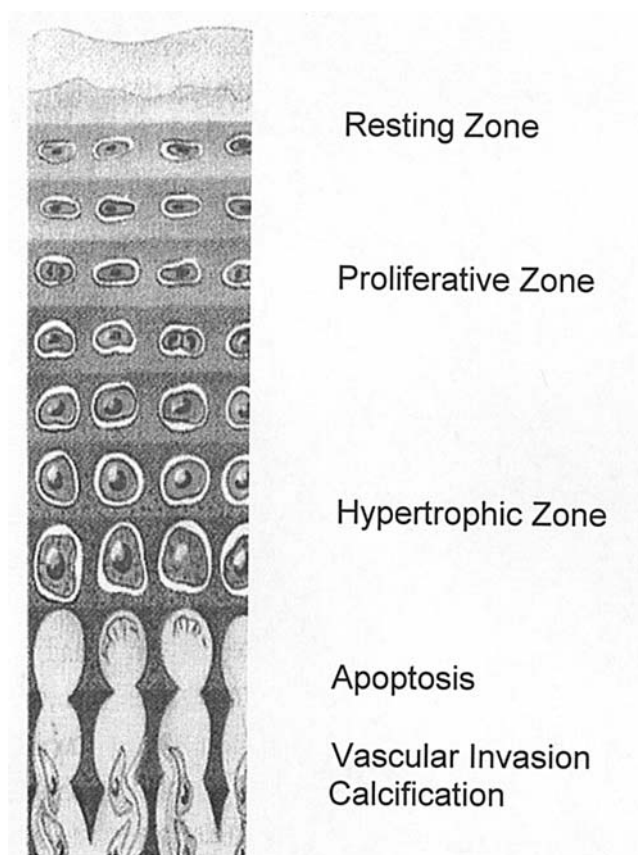
Figure 1. Chondrocyte maturational phases within the growth plate.

The growth plate chondrocytes are in columns in the direction of the bone's longitudinal axis. Longitudinal and transverse septae consisting of ECM divide each column and each chondrocyte inside the columns respectively. The ECM of the growth plate determines its mechanical properties. The ECM also offers a scaffold for chondrocyte adhesion and movement, thereby contributing to the growth plate's histological design (43). During terminal differentiation, mineralisation of the ECM surrounding the hypertrophic chondrocytes occurs. Functionally the ECM changes to an environment permitting vascular invasion, allowing osteoclasts and differentiating osteoblasts to remodel the new cartilage into bone tissue (44).

In order to compensate for the rapid chondrocyte proliferation and hypertrophy rates, the differentiated chondrocyte must be removed in order to maintain a constant growth plate width. In growing rats, it has been determined that eight hypertrophic chondrocytes are removed each day by apoptosis (39). However, the growth plate tapers and ultimately disappears near the end of the growth phase. The control of growth termination is central to the growth plate, with the cessation of growth preceeding rather than following growth plate fusion (45,46).

GH and IGF-1 are two of the most significant and widely investigated regulators of post-natal bone growth, and exert direct effects on the growth plate. A dual effector theory of GH/IGF-1 action at the level of the growth plate has been proposed, whereby GH acts directly on germinal zone precursors of the growth plate to induce chondrocyte differentiation, with a subsequent increase in local IGF-1

synthesis, which in turn results in the clonal enlargement of chondrocyte columns in an autocrine/paracrine mechanism (47,48). However, chondrocytes in all of the growth plate's maturational zones express IGF-1, with IGF-1 mRNA expression predominantly limited to the hypertrophic zone. *In vivo* studies involving IGF-1 infusion of hypo-physectomised rats, show stimulation of chondrocytes in all maturational zones, including the hypertrophic (49-51). Growth plate-derived IGF-1 is considerably more important for post-natal growth compared to serum IGF-1 (52,53).

Parathyroid hormone-related peptide (PTHrP) plays an important role in locally controlling the cellular function of growth plate chondrocytes. Mice missing the PTH/PTHrP receptor gene, and mice that are homozygous for the ablation of the PTHrP gene have comparable growth plate morphologies (54). Accelerated chondrocyte differentiation and early mineralisation produce the thin growth plate observed in these mice. Conversely, mice overexpressing the PTHrP gene show a remarkable deceleration of chondrocyte differentiation and a larger growth plate (55). PTHrP, together with the morphogen Indian hedgehog (Ihh), exert important effects on bone growth (38).

Sex steroids induce direct effects on the growth plate, and are extremely important in the control of endochondral growth. Androgen receptor, estrogen receptor α (ERα) and estrogen receptor ß (ERß) mRNA and protein expression have been observed in growth plate tissue (56). This demonstrates that androgens and estrogens directly control growth plate cellular function. The growth plate also has the capacity for steroidogenesis and aromatisation (57). However, it has been difficult to determine whether androgens directly influence the growth plate cartilage. Nonaromatisable androgens, such as dihydrotestosterone, control both chondrocyte proliferation and differentiation, by inducing local IGF-1 synthesis and increasing IGF-1 receptor expression (58,59). In both sexes, estrogen is the essential hormone in regulating growth plate acceleration and fusion (60,61). Estrogen changes proliferation, alkaline phosphatase activity and proteoglycan synthesis in chondrocytes (62,63). Interestingly, growth plate chondrocyte proliferation is increased by low levels of estrogen and reduced by high levels (64).

Pro-inflammatory cytokines are involved in bone development, in the initiation and control of skeletal tissue growth, and in regulating bone remodelling (65). Both TNF and IL-1ß may act at the level of the growth plate. TNFα induces neo-vascularisation *in vivo*, and may be involved in the stimulation of growth plate vascular invasion (66). IL-1ß mRNA has been localised in the calcified cartilage zone of the growth plate, and together with bone morphogenetic protein (BMP) enhances cartilage formation *in vitro* (67). However, whilst cytokines play important roles in bone development, the elevated concentrations of pro-inflammatory cytokines associated with JIA and IBD may lead to detrimental effects on bone growth through systemic effects and/or a local effect at the level of the growth plate.

4. Systemic effects of cytokines through the GH/IGF axis

Elevated levels of circulating IL-6 have been observed in children suffering from JIA and IBD (68,69). These patients
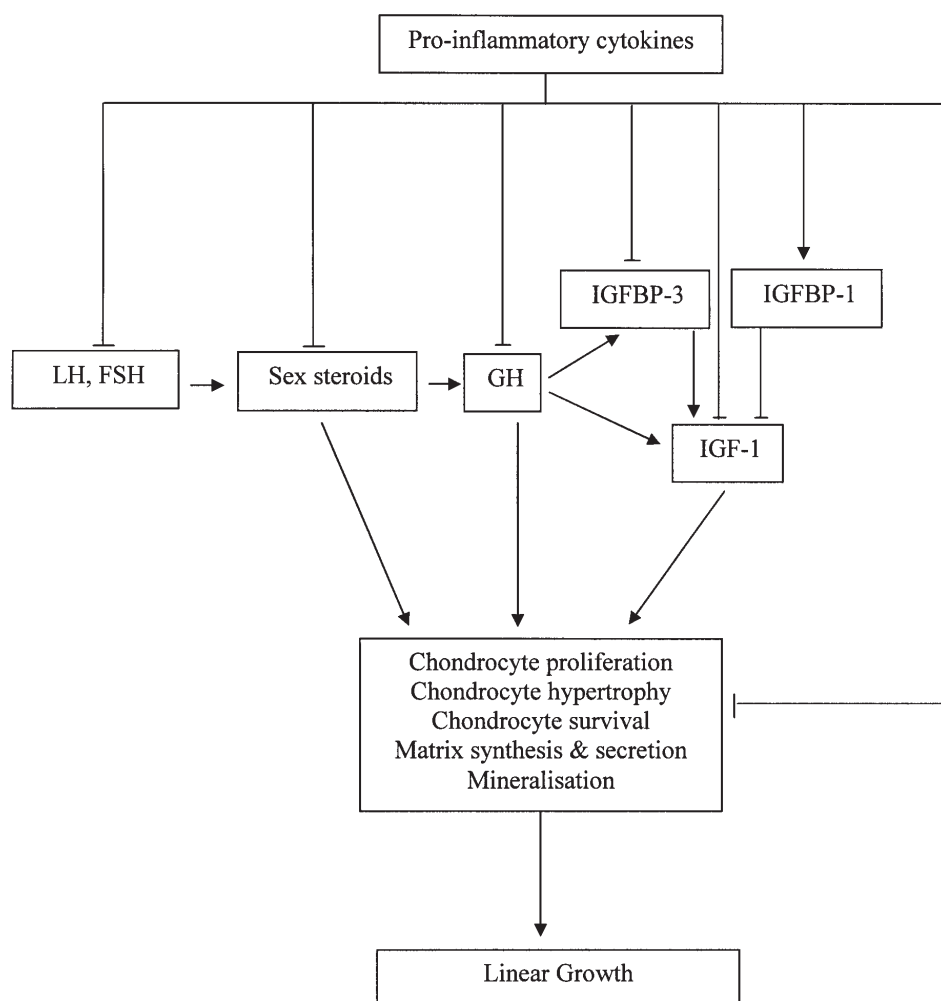
Figure 2. Possible mechanisms underlying pro-inflammatory cytokine-mediated growth retardation at a systemic and local level.

show reduced circulating IGF-1 levels, with unchanged GH levels (70,71). Low concentrations of IGF binding protein-3 (IGFBP-3) may also be seen in these patients (70-72). The mechanisms by which the pro-inflammatory cytokine IL-6 causes systemic effects on growth have been examined using the NSE/hIL-6 transgenic murine model, which overexpresses IL-6. Elevated circulating IL-6 and growth retardation are observed in these mice (73), however growth is normalised following IL-6 neutralisation (74). Like the JIA and IBD patients, these transgenic mice have reduced circulating IGF-1 levels, with unchanged GH (73), and also reduced IGFBP-3 levels (72). It is probable that IL-6 reduces IGF-1 levels by increased clearance. This is because the association of IGF-1 in the circulating ternary complex with IGFBP-3 and an acid-labile subunit (ALS) markedly prolongs the half-life of IGF-1 (72).

The pro-inflammatory cytokine TNFα is also associated with JIA and IBD (7,75). TNF-transgenic mice, which overexpress TNFα, show growth retardation (76,77). However, alterations in the IGF-1/GH axis have yet to be examined in this transgenic model.

IL-1ß treatment reduces plasma levels of both IGF-1 and ALS (78-80). Hepatic IGFBP-1 expression is increased in septic rats, which inhibits IGF-1 bioactivity, as phosphorylated IGFBP-1 has a higher affinity for IGF-1 than the

IGF-1 receptor (81,82). This effect can be completely reversed by treatment with an IL-1 receptor antagonist (82). IL-1ß also increases IGFBP-1 protein and mRNA synthesis in the HepG2 hepatoma cell line (83-85).

5. Local effects of cytokines on the growth plate through the GH/IGF axis

The effect of IL-6, IL-1ß, and TNFα on chondrocytes has been comprehensively studied *in vitro*. However, investigations have predominantly focused on the effects of cytokines on articular chondrocytes, rather than on growth plate chondrocytes.

Localised damage to the growth plate of long bones is associated with inflammatory synovitis, indicated by increased concentrations of synovial IL-6, TNFα and IL-1ß (86). This suggests that the cytokines in the synovial fluid can act directly on the growth plate from the adjacent synovial space.

The effect of IL-6 on articular chondrocyte differentiation has been described. It has been shown that the stimulatory effect of IGF-1 on proteoglycan synthesis is reduced by IL-6 (87). In bovine articular chondrocytes, IL-6 with the addition of soluble IL-6 receptor also inhibits the expression of gene markers of chondrocyte differentiation, including aggrecan,

type II collagen and link proteins (88). IL-6 has been shown to have no effect on growth plate chondrocyte dynamics (89), however these experiments were performed without the addition of the soluble IL-6 receptor.

Both IL-1ß and TNFα have been shown to inhibit growth plate chondrocyte differentiation (90-94). IL-1ß and TNFα have also been shown to increase DNA synthesis in growth plate and costal chondrocytes (95-97), which may be the mechanism for the excessive endochondral bone growth observed in the affected limbs of children with oligoarticular JIA. TNFα has been shown to cause apoptosis in chick chondrocyte cultures (98) and to inhibit cartilaginous nodule formation and the production of cartilage-specific proteoglycans in ATDC5 murine growth plate cells (99). TNFα has also been shown to inhibit proteoglycan production in foetal mouse metatarsals (100). This study also showed that IL-17 synergises with TNFα to further inhibit proteoglycan synthesis. IL-1ß also synergises with TNFα to inhibit endochondral growth in foetal rat metatarsal bones (89).

The cellular mechanisms through which cytokines act on the growth plate have yet to be elucidated. Possible mechanisms include increased cell apoptosis induced by TNFα (89,99), the down-regulation of Sox9 gene expression (101), a master regulatory factor for chondrocyte differentiation and cartilage formation (102), effects on the growth plate through the gonadal axis (105-107), inhibition of IGF-1 signalling (106-108) and interference with GH signalling (109).

The IGF-1 signalling pathway is a major autocrine/paracrine controller of endochondral bone growth (110). IGF-1 exerts its cellular effects through a receptor tyrosine kinase (IGF-1R), which is expressed in growth plate chondrocytes. However, studies have shown that neither IL-1ß nor TNFα reduce IGF-1R expression (111), alter IGF-1R affinity (112) or inhibit the intrinsic tyrosine kinase activity of the IGF-1R (113,114). It is therefore doubtful that inflammatory cytokines affect the IGF-1 signalling cascade at the IGF-1 receptor level.

IGF-1 receptor binding initiates an IGF-1 signalling cascade, which may be altered by pro-inflammatory cytokines at one or more points: insulin receptor substrate (IRS) phosphorylation; the Erk1/Erk2 mitogen-activated protein kinase (MAPK) signalling pathway; the phosphatidylinositol 3-kinase (PI-3K) signalling pathway and Akt phosphorylation. The effects of pro-inflammatory cytokines on IGF-1 signalling in growth plate chondrocytes at these junctures have yet to be determined. However, effects of cytokines on IGF-1 signalling have been investigated in a number of other cell types. TNFα and IL-1ß inhibit IRS-1 phosphorylation in both myoblasts (106,114) and breast cancer epithelial cells (113). TNFα inhibits MAPK-kinase phosphorylation in neuronal cells (107), prevents the intranuclear translocation of PI-3K in osteoblasts (108) and inhibits Akt phosphorylation and activation in neuronal cells (107).

Increased concentrations of pro-inflammatory cytokines may also alter the effects of GH on the growth plate. Cytokines may interfere with GH receptor signalling (109), although little research has been performed in this area. IL-1ß and TNFα induce the expression of SOCS (suppressor of cytokine signalling proteins) (115). SOCS are cellular signalling proteins that down-regulate cytokine signalling and alter GH signalling (116,117). Mice genetically modified to not express SOCS2 show gigantism accompanied by deregulated GH signalling (118). Furthermore, IL-6 may reduce liver GH signalling by inducing SOCS3 (119), a mechanism that may explain GH resistance observed in inflammatory diseases.

6. Conclusions

Pro-inflammatory cytokines may modulate growth patterns in children with inflammatory diseases such as Juvenile Idiopatic Arthritis and Inflammatory Bowel Disease through both systemic and local effects of the GH/IGF-1 axes (Fig. 2). By elucidating the fundamental cellular mechanisms that are altered in growth plate development, we will be in a better position to treat the abnormal growth patterns observed in affected children.

Acknowledgements

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