ABSTRACT

Growth disorders are common among patients with juvenile idiopathic arthritis (JIA). These disorders range from general growth retardation to local acceleration of growth in the affected limb, and are associated with an increased production of pro-inflammatory cytokines, such as interleukin-1β (IL-1β), tumour necrosis factor-α (TNF-α) and interleukin-6 (IL-6). Pro-inflammatory cytokines may act individually or in combination to impair child growth through systemic mechanisms and/or a local action. Whereas IL-6 affects growth mainly via systemic mechanisms altering growth hormone secretion, IL-1β and TNF-α can directly affect growth plate chondrocyte dynamics as well as longitudinal bone growth. There are emerging data suggesting that interleukin-15 and interleukin-5 may be new cytokines implicated in inflammatory diseases, but further well-designed longitudinal studies in larger groups of children are required to establish a causal relationship. Other factors, which might contribute to growth suppression associated with childhood arthritis, include the degree, extent, and duration of disease activity, age at onset, immobility, sub-optimal nutrition and corticosteroid therapy.

Introduction

Juvenile idiopathic arthritis (JIA) is a chronic inflammatory disease affecting the joints and represents the most common chronic rheumatic disease during childhood (1). Between 5 and 18 per 100,000 children develop JIA each year, and the overall prevalence is approximately 30–150 per 100,000 (2). Growth is often impaired in children with chronic inflammatory diseases such as juvenile idiopathic arthritis (JIA) (3). These disorders are characterised by growth alterations ranging from general growth retardation to local acceleration of growth in the affected limb (4). The degree of growth failure is variable and includes mild decreases in growth velocity as well as severe forms of short stature (4). Several factors can impair growth, such as chronic inflammation, corticosteroid treatment, under-nutrition, and reduced physical activity (5). These factors can act on the growth hormone (GH)-insulin-like growth factor-1 (IGF-1) axis, altering its function at one or more levels, centrally or peripherally (4). Delays in pubertal onset and/or a slow pubertal progression are often associated with growth impairment in young patients with chronic diseases (5).

In this review we report the current knowledge on growth disorders in children with JIA, with particular reference to the role of inflammatory cytokines as potential mediators of growth impairment.

Growth in juvenile idiopathic arthritis

Human growth is a complex phenomenon depending on the interaction of several players, including genes and environmental factors, such as nutrition, as well as hormones and growth factors. GH is the main mediator of somatic growth during postnatal life, mainly through its peripheral mediator, IGF-1 (6). Several studies have assessed growth patterns in children and adolescents with JIA as well as in animal models of JIA and tried to understand the underlining mechanisms (3, 7).

In JIA the prevalence of short stature ranges from 10.4% in children with the polyarticular form to 41.0% in patients with the systemic form (8, 9). To define patterns of growth in JIA, Liem et al. examined 67 children with different forms of JIA followed for a period ranging from 5 to 18 years (10). The group with the pauciarticular form of JIA showed relatively stable height velocity throughout the follow-up period. In contrast, the group with the systemic form manifested a progressive
decline in height velocity over time. The group with the polyarticular form had a modest decline in height velocity during the first 5 years of follow-up, followed by a tendency towards normalisation (10). Overall these results indicate that heights-for-age values in the polyarticular group tend to be lower than those in the pauciarticular group although not as low as in the systemic population (10). These data support the observations of Zak et al. (11), who assessed final height in 65 adults with chronic childhood arthritis. Patients with a height less than 2 standard deviations below the mean (10.8%) tended to have polyarticular or systemic onset subtypes, exposure to systemic steroid therapy, and more functional disability.

Factors which might contribute to growth suppression associated with childhood arthritis include the degree, extent and duration of disease activity, age at onset, immobility, sub-optimal nutrition, defects in GH secretion and action, corticosteroid therapy and the effect of pro-inflammatory cytokines (10). With regards to therapy, significant deviations of adult height from mid-parental height have been reported only in children treated with systemic steroids for longer than 12 months (12). In a retrospective study of 24 children with systemic JIA (9), 87% of them had a final height less than their target height. Furthermore, no catch-up growth was observed in 30% of children following disease remission and termination of glucocorticoid therapy.

Previously, among 119 children with JIA, Ansell and colleagues (13) observed that growth retardation was associated with a long duration of active disease, but that disease remission was associated with improvement in growth velocity, leading to achievement of a normal height after 2 to 3 years, unless epiphyseal growth nuclei had already fused prematurely (13). Similarly, in a population of 64 prepubertal children with chronic juvenile arthritis, Saha et al. found an initial decrease in height during the first year post-diagnosis followed by an increase, suggesting that growth can normalise to pre-disease values with a good control of the disease activity (14). In children with JIA, maintenance of growth is also influenced by a number of systemic and local autocrine/paracrine factors, including vitamin D metabolites, sex steroids, parathyroid hormone-related peptide, fibroblast growth factor, bone morphogenic proteins together with other members of the transforming growth factor superfamily (15).

Role of cytokines in growth impairment in JIA

There is growing evidence suggesting that cytokines can contribute to abnormal growth patterns in children with JIA. Cytokines, which have been reported to play a major role in JIA, include interleukin-1β (IL-1β), tumour necrosis factor-α (TNF-α) and interleukin-6 (IL-6) (Table I) (16). TNF-α and IL-1β have the potential to directly affect growth plate chondrocyte dynamics, whereas IL-6 may influence growth by reducing also the systemic effects of IGF-1 (17, 18). The action of these cytokines is exerted via specific cell surface receptors resulting in changes in gene expression of target cells. Receptors of different cytokines share common signal transduction pathways allowing cross-over effects. Pro-inflammatory cytokines may act individually or in combination to affect child growth through systemic mechanisms and/or a local action at the level of the growth plate of long bones (4).

Interleukin-6

IL-6 is a pleiotropic cytokine that regulates immune response, haematopoiesis, acute-phase response and inflammation (19). In animal models of chronic inflammatory disease, such as IL-6 transgenic murine lines, IL-6 appears to be the main pro-inflammatory cytokine involved in growth retardation (20).

Circulating and synovial fluid levels of IL-6 are markedly elevated in patients with systemic JIA, and they are associated with laboratory and clinical variables of disease activity (21). An association has also been observed between inflammatory clinical activity and growth retardation (8, 10). Souza and colleagues (3) showed an inverse association between growth velocity and serum IL-6 levels as well as disease activity in 79 patients with JIA followed for 1 year. In particular, they found that the prevalence of reduced growth velocity was 25.3%. Twelve (60%) of these 20 patients presented levels of IL-6 >1pg/ml, whereas out of the 58 patients with normal growth velocity, only 16 (27.6%) showed levels of IL-6 >1pg/ml (3).

Given that the polyarticular and systemic forms are usually associated with a higher inflammatory activity, and taking into account that elevated IL-6 levels were associated with lower growth velocity Z-scores, Souza et al. suggested that IL-6 could be an essential link between inflammation and stunted growth (3).

Studies performed in transgenic murine models have examined the potential mechanisms by which IL-6 induces systemic effects on growth retardation. De Benedetti et al. (22) found that NSE/hIL-6 transgenic mice lines expressing high levels of circulating IL-6 since soon after birth presented a reduced growth rate that led to a mice size reduction of 50–70% when compared to nontransgenic littermates. Administration of a monoclonal antibody against the murine IL-6 receptor partially reverted the growth defect. In NSE/hIL-6 transgenic mice, circulating IGF-1 levels were lower than those of nontransgenic littermates; whereas, the distribution of GH pituitary cells, as well as circulating GH levels, were normal (Fig. 1) (22).

NSE/hIL-6 mice and wild-type mice treated with IL-6 showed decreased levels of IGF-binding protein-3 (IGFBP-3) (7), the main IGF-1 binding protein, which together with the acid-labile subunit (ALS) and IGF-1, forms the ternary complex, which increases the half-life of circulating IGF-1 (23). Patients with systemic JIA, condition characterised by a high IL-6 production, also show markedly reduced levels of IGFBP-3. The mechanism by which IL-6 induces decreased IGFBP-3 levels remains to be clarified. Whereas IGF-1 and ALS are produced by hepatocytes and their production is not affected by IL-6, IGFBP-3 is produced by Kupffer cells.
cells (24). As Kupffer cells constitutively express IL-6 receptor, it can be hypothesised that IL-6 directly affects IGFBP-3 production. On the other hand, De Benedetti et al. (7) showed that both NSE/hIL-6 transgenic mice and patients with systemic JIA have increased proteolysis of serum IGFBP-3, suggesting that IL-6-induced decrease in intact IGFBP-3 levels is, at least partly, due to IGFBP-3 proteolysis. This could be related to the known ability of IL-6 to stimulate the production of several proteases including cathepsin B and L (25) and, more interestingly, of metalloproteinases (MMP) from a variety of cell types (26).

Low levels of IGFBP-3 are associated with a marked decrease in IGF-1 half-life and accelerated clearance leading to low IGF-1 levels even in the presence of normal liver IGF-1 production (27). IGFBP-3 levels were directly correlated with IGF-1 levels both in NSE/hIL-6 mice and in patients with systemic JIA (7).

Generally, IL-6 has not been considered to have any direct effect on growth plate chondrocyte dynamics but to affect growth only through systemic mechanisms that alter GH/IGF-1 axis (7, 28). However, a recent study demonstrates that IL-6 directly inhibits early differentiation of growth plate chondrocytes, and that growth impairment in systemic JIA may be in part due to a direct inhibitory effect of IL-6 on committed stem cells in the growth plate (29). Nakajima et al. (29) evaluated the effects of IL-6 on chondrogenesis in ATDC5 cells, a clonal murine chondrogenic cell line that provides an excellent model for studying endochondral ossification at growth plate. At the epiphyseal end of the growth plate, the germinial or stem cell zone contains the resting chondrocytes (30). Owing to some unknown triggers, the stem cells enter the proliferating zone, and local and systemic factors regulate longitudinal bone growth, which involves the differentiation of committed stem cells into proliferating chondrocytes (early chondrogenesis); after a certain number of cell divisions, these cells finally differentiate into the hypertrophic phenotype that deposits a matrix that is mineralised and eventually replaced by bone (31). In the assessed murine chondrogenic cell line (ATDC5), IL-6 inhibited cartilaginous nodule formation, an important prerequisite for initiation of chondrogenesis in mesenchymal cell cultures, (29) as well as markedly reduced the expression of type II collagen, aggrecan and type X collagen (Fig. 2) (29). These data support a negative role of IL-6 in the early differentiation of chondrocytes, although these findings are limited to a specific murine cell lines.

De Benedetti et al. (32) evaluated the effects of chronic IL-6 over-expression in vivo, using the skeletons of growing prepubertal mice exposed to high circulating IL-6 levels since birth. Their study showed that during prepubertal development, IL-6 over-expression caused osteopenia, with accelerated bone reabsorption, reduced bone formation, and defective ossification, pointing to IL-6 as a pivotal mediator of the damage induced by chronic inflammation in postnatal bone development (32, 33). Enhanced bone resorption was due to increased osteoclastogenesis, and reduced osteoblast activity was induced by a mechanism affecting precursor proliferation and osteoblast function (32). Osteoblast differentiation appeared to be unaffected, as shown by unaltered Runx2 and alkaline phosphatase mRNA expression. The discrepancy between unchanged Runx2 expression and low expression of its downstream gene osteocalcin may be explained by possible modulation of Runx2-specific coactivators/repressors by IL-6 (34). The activation of osteoclasts in IL-6-transgenic mice, instead, was not mediated by an increased production of these cytokine by the osteogenic cells or by increased circulating levels that were not found to be changed in vivo (32, 35).

Nakajima et al. (36) assessed skeletal abnormalities in 201 healthy children with JIA aged 0-16 years, by measuring serum cartilage oligomeric matrix protein (COMP) concentrations, a protein produced mainly by chondrocytes and synovial cells. In children, serum COMP concentrations apparently reflect chondrocytes turnover in the growth plate. Their study revealed that serum COMP levels in patients with systemic JIA were decreased during active disease phase whereas its levels significantly increased after treatment with the anti-IL-6 tocilizumab (36). In the same study, serum MMP-3 concentration, which is a useful marker for predicting joint destruction and for disease activity in rheumatoid arthritis (37), and serum bone alkaline phosphatase (BAP) concentration, which is one of the bone formation markers for osteoblastic activity (38), were also assessed. MMP-3 concentrations were high during the active disease phase, whereas decreased after starting treatment with tocili-
zymab (36). In contrast, serum BAP concentrations were low during active disease and then significantly increased during the remission phase, similarly to COMP, suggesting that growth cartilage and bone turnover may be improved as a result of the successful inhibition of inflammation under treatment with anti-IL-6 (36).

**Tumour necrosis factor-α and interleukin 1-β**

In contrast to IL-6, which affects growth mainly via systemic mechanisms that alter GH secretion, IL-1β and TNF-α can directly affect growth plate chondrocyte dynamics as well as longitudinal bone growth (18).

These cytokines inhibit the expression of a number of genes encoding chondrocyte-specific matrix molecules, including aggrecan, collagen types IX and XI (39, 15). Murine ATDC5 chondrogenic cells and postnatal metatarsals, exposed to interleukin IL-1β and TNF-α showed reduced chondrocyte proliferation and expression of aggrecan and collagen types II and X (17). Cytokine exposure particularly inhibited proteoglycan synthesis. Proteoglycans and, in particular, aggrecan together with collagen type II, provide a scaffold and stimuli for chondrocyte attachment, migration and differentiation and, thereby, maintain the structural organisation of the growth plate (Fig. 2) (40).

In a study by MacRae (17), IL-1β and TNF-α led to a 71% and 45% reduction, respectively, in metatarsal growth after 8 days of exposure. An additive effect of IL-1β and TNF-α was observed (110% decrease). Their combination led to growth being 59% lower compared with control metatarsals at the end of the recovery period. Exposure to the combination for 4 days followed by a 4-day recovery period resulted in 87% decrement compared with controls (17).

Similar data were previously reported by Mårtensson and coworkers (18) who studied the effects of cytokines on longitudinal bone growth in fetal rat metatarsal bones kept in culture. After a 7-day culture, bones were sectioned and chondrocyte proliferation was assessed. When added separately, IL-1β and TNF-α impaired longitudinal bone growth only at a high concentration (100ng/ml each). In contrast, when added in combination, IL-1β and TNF-α potently inhibited growth at lower concentrations (from 3ng/ml each). These effective concentrations of IL-1β and TNF-α are supraphysiological compared with the serum levels reported in normal children but not far from those reported in children with JIA (41). A possible explanation for the additive effect of these cytokines could be that these cytokines share some mitogen-activated protein kinase (MAPK)-signalling pathways and that both cytokines are needed to evoke effects (42). In addition, in many inflammatory diseases associated with growth retardation, more than one cytokine is upregulated, suggesting that growth retardation could be a result of the synergistic effect of two or more cytokines (16).

IL-1β and TNF-α also decreased chondrocyte proliferation and increased apoptosis (Fig. 2) (16). Apoptotic cells were almost exclusively proliferative chondrocytes with almost none of the hypertrophic cells affected.
These cytokines induced loss of early chondrocytes and depleted the growth plate of cells that are programmed to give longitudinal growth (16).

The results of these studies concur with and extend previous reports highlighting the effects of inflammatory cytokines on growth plate chondrocytes. In cultures of rabbit growth plate chondrocytes, IL-1β decreased alkaline phosphatase activity during the hypertrophic stage and suppressed an increase in cell size and type X collagen expression, suggesting an inhibition of chondrocyte terminal differentiation and hypertrophy (43). TNF-α induced apoptosis in chick growth plate chondrocyte cultures (44) and suppressed cartilaginous nodule formation and the accumulation of cartilage specific proteoglycan reduction in the ATDC5 cell line (45). Interestingly, Horiguchi et al. (45) noted that TNF-α (10ng/ml) increased (3H)thymidine uptake in the ATDC5 cell line which is in contrast to the data of MacRae et al. (17). The reason for this discrepancy is unclear; however, the reduction in chondrocyte proliferation noted in the ATDC5 cell line was confirmed in the metatarsal model. TNF-α has also been shown to synergise with IL-17 to reduce proteoglycan synthesis in fetal mouse metatarsals (46).

Several studies have suggested that longitudinal growth and especially chondrocyte hypertrophy in the metatarsal model of growth is highly dependent on IGF-1 action (47,48).

Proinflammatory cytokines may modulate the IGF-1 signalling pathway in growth plate chondrocytes at several levels (15). It is unlikely that IL-1β and TNF-α down-regulate IGF-1 receptor expression (15,49), affect IGF-1 receptor affinity (50) in articular cartilage or inhibit the intrinsic tyrosine kinase activity of the IGR-1 receptor (IGF-1R) (51,52). It has been shown that TNF-α and IL-1β induce IGF-1 resistance by inhibiting insulin receptor substrate-1 (IRS-1) phosphorylation in both myoblasts (53) and breast cancer epithelial cells (51). Proinflammatory cytokines may therefore also induce IGF-1 resistance by inhibiting IRS-1 phosphorylation in growth plate chondrocytes (15).

However, proinflammatory cytokines may also act directly on the Phosphoinositide 3-kinase (PI-3K) and/or MAPK signalling pathways of growth plate chondrocytes (54). At this regard, MacRae et al. (55) studied the relationship between IL-1β and IGF-1, by adding the inhibitors of the PI3K (LY294002) and Erk 1/2 (PD98059) to ATDC5 chondrocyte cell line and murine fetal metatarsal cultures. IGF-1 stimulated ATDC5 chondrocyte proliferation. Addition of PD98059 or LY294002 supplemented ATDC5 cultures partially reduced proliferation by 32%, and 66%, respectively. Total inhibition of IGF-1 stimulated ATDC5 proliferation was only observed in the presence of both inhibitors (55).

Finally, TNF-α and IL-1β may also directly interfere with GH receptor signalling. The effects of GH are generally manifested through its interaction with a specific cell surface receptor, the GH receptor (GHR) (56). GH binding to the GHR promotes the activation of the intracellular tyrosine kinase JAK2 (57). GH also stimulates tyrosyl phosphorylation of the GHR and several cellular proteins, including MAPK, extracellular signal-regulated kinase 1 and 2, and the signal transducers and activators of transcription (Stat) 1, 3 and 5, probably through pathways involving JAK kinases (58). Phosphorylated Stat proteins form multimeric complexes that bind specific DNA elements and directly regulate transcription of responsive genes (59). The peripheral mediator of GH, IGF-1, acts through a tyrosine kinase receptor (IGF-1R). Binding of IGF-1 to its receptor activates intracellular substrates, mainly IRSs, and a series of autophosphorylation events (60).

There is evidence that TNF-α and IL-1β can increase suppressor of cytokine signalling proteins (SOCS)-3 expression, and this directly inhibits IRS-1 phosphorylation by IRS-1 protein degradation (61). SOCS are cellular signalling proteins that down-regulate cytokine signalling and alter GH signalling (62). SOCS-2 knock-out mice show giantism accompanied by deregulated GH signalling (63).
the potential mechanisms behind the reported associations.

Conclusions

Growth retardation is often a major problem in patients with JIA and can result in a short final stature of variable degree, especially when the disease is severe. There is evidence that pro-inflammatory cytokines contribute to abnormal growth patterns in children with JIA through systemic and local effects on the GH/IGF-1 axis. The studies commented in this review highlight the concept that new therapeutic strategies in children and adolescents with JIA might consist in the development and application of cytokine inhibitors, such as anti-TNF monoclonal antibodies or humanised anti-IL-6 receptor monoclonal antibody, not only to contrast the disease but also to improve the growth disturbances often associated.

References

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