The role of interleukin-6 in rheumatoid arthritis-associated osteoporosis

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Abstract

Introduction Osteoporosis is highly prevalent in patients with rheumatoid arthritis (RA) and is a frequent cause of fractures, disability, reduced quality of life and increased use of healthcare resources.

Discussion Factors associated with the development of osteoporosis and fractures in patients with RA include disease activity, inflammation, gender, age, low body mass and glucocorticoid exposure. Several processes contribute towards the pathology of RA-associated osteoporosis, and increased osteoclast activation and subsequent bone resorption mediated by pro-inflammatory cytokines are thought to play major roles. Given the key effects of interleukin-6 (IL-6) in both RA and osteoporosis, and its ability to modulate other inflammatory mediators, IL-6 may be an important factor specifically associated with osteoporosis in patients with RA.

Conclusion The development of agents that modulate the actions of IL-6 and those of other pro-inflammatory mediators of bone loss may provide alternative osteoporosis management strategies for patients with RA than existing general osteoporosis therapies.

Keywords Cytokines · Interleukin-6 · Osteoporosis · Rheumatoid arthritis

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease that primarily manifests in the synovial joints.

Synovial inflammation, pannus formation, cartilage degradation and focal bone erosions are common features of joints affected by RA [1, 2]. However, RA is a systemic illness with a host of associated extra-articular comorbidities, including osteoporosis, anaemia, lymphoproliferative malignancy and cardiovascular disease [3, 4].

Osteoporosis is one of the most common comorbidities in patients with RA, and has been reported in 10–56% of patients, depending on the populations studied [5, 6]. As a consequence, patients with RA are at substantially higher risk of suffering fracture compared with the general population. In a retrospective study of 30,262 patients, van Staa et al. [7] showed that patients with RA had an increased risk of fractures at both the hip (risk ratio [RR]=2.0) and spine (RR=2.4) compared with controls. The risk was markedly increased by the use of corticosteroids (RR=3.4). In another investigation, approximately one third of women with RA treated with prednisolone reported a fracture within 5 years [8]. Despite the evident association between glucocorticoid use and bone loss in RA as well as in other conditions that are treated with glucocorticoids, bone loss is also common in patients with RA who have never been treated with glucocorticoids [9, 10] and these patients also have an increased risk of fracture [7]. It is clear that in RA, factors other than glucocorticoid use are also important in the pathogenesis of osteoporosis.

Pro-inflammatory cytokines including tumour necrosis factor (TNF)-α, interleukin (IL)-1 and IL-6 are key mediators of the multiple articular and extra-articular manifestations of RA [11–15]. The involvement of IL-6 in RA and associated comorbidities may reflect its ability to affect the function of a number of different cell types, including neutrophils, T cells, B cells and monocytes [16]. Importantly, IL-6 also appears to be one of the central mediators of osteoclast activity, supporting a crucial role in
osteoarthritis. Given the key effects of IL-6 in both RA and osteoporosis, and its ability to modulate other inflammatory mediators, IL-6 may be an important factor in RA-associated osteoporosis. This article will discuss the role of cytokines, particularly IL-6, in the pathophysiology of osteoporosis and the potential for using targeted therapies for the treatment of osteoporosis in patients with RA.

Risk factors and consequences of osteoporosis in patients with RA

Generalised osteoporosis and fractures are significant complicating factors in RA. Systemic bone loss of up to 10% has been observed in early RA and an increased risk of both hip and pelvic fractures has been demonstrated [7, 17, 18]. The high risk of osteoporosis in patients with RA may be due to a number of disease-specific factors, including disease activity, medication effects and physical inactivity, as well as general risk factors, such as postmenopausal status and increased age [19]. The causes of osteoporosis may also be interrelated, such that specific treatments for RA, for example glucocorticoids, may increase susceptibility to osteoporosis, but also decrease inflammatory activity, which is associated with bone loss [20]. Previously, studies investigating risk factors for osteoporosis in patients with RA have been conducted primarily only in women or even only in postmenopausal women. However, a recent study investigated factors predisposing to osteoporosis in 343 postmenopausal women, 100 premenopausal women and 108 men with RA [21]. Osteoporosis was found in a significantly higher percentage of postmenopausal women (55.7%) and in men (50.5%) compared with premenopausal women (18.0%; p<0.001). Risk factors for osteoporosis were dependent on gender and menopausal status such that older age, lower body mass index (BMI) and higher cumulative glucocorticoid dose were significant risk factors in postmenopausal women, lower BMI and higher cumulative glucocorticoid dose were significant risk factors in men, and lower BMI was a significant risk factor in premenopausal women. Additional studies have sought to determine factors associated with risk of fractures in patients with RA and osteoporosis [8, 13, 22]. In patients with RA, multivariate analyses identified years taking prednisone, previous diagnosis of osteoporosis, disability, age, lack of physical activity, female sex, disease duration, impaired grip strength and low body mass as independent predictors of fractures [8]. Not only does the risk of fractures and increased fragility impact on an individual's quality of life (QoL), there are also considerable societal costs. For instance, following a hip fracture, there is a 10–20% mortality rate over the subsequent 6 months, 50% of sufferers will be unable to walk without assistance and 25% will require long-term domiciliary care [23, 24].

Pathology of osteoporosis in patients with RA

Mechanisms of bone loss

Bone loss can occur through suppression of bone formation, increased bone resorption, or a mixture of both. The predominant effect of glucocorticoids appears to be on bone formation [25], but in patients with early RA, who have not been treated with glucocorticoids, studies correlating markers of bone turnover with bone mineral density (BMD) suggest that increased bone resorption by osteoclasts is the dominant mechanism [26]. This is consistent with a major role for pro-inflammatory cytokines in driving osteoporosis in RA, given that many pro-inflammatory cytokines are involved in osteoclastic differentiation and activation, including IL-6, IL-1, TNF-α and transforming growth factor-β, and their actions are often synergistic and interrelated. TNF-α and IL-1 are secreted by macrophage-like cells and can induce IL-6, which activates bone resorption pathways and is a major activator of the hepatic acute-phase response. These cytokines are also key regulators of receptor activator for NFκβ ligand (RANKL) and osteoprotegerin (OPG), themselves important mediators of osteoclast differentiation and activation [15]. Increased levels of circulating cytokines correlate with disease progression and activity.

It is widely recognised that IL-6 is a potent stimulator of osteoclast-induced bone resorption and central to the pathogenesis of bone loss in the context of chronic inflammation [27]. IL-6-overexpressing transgenic mice exhibit osteopenia with severe alterations in cortical and trabecular bone microarchitecture, as well as uncoupling of bone formation from resorption, with decreased osteoblast and increased osteoclast number and activity [27]. In addition, IL-6 appears to mediate, at least in part, the bone resorption-inducing effects of TNF-α and IL-1, because neutralising monoclonal anti-IL-6 antibodies can suppress TNF- or IL-1-stimulated osteoclast development [13, 28]. Molecular studies have shown that IL-6 mRNA is expressed significantly more often in bone samples from postmenopausal women with osteoporotic fractures than in women with normal BMD or postmenopausal women on hormone replacement therapy (94%, 51% and 40%, respectively, p<0.01) [29]. A study in 193 healthy men and women showed an association between BMD and circulating markers of inflammation, with IL-6 most strongly predictive for loss of BMD [30].
Receptor mechanisms and IL-6

The actions of IL-6 are mediated through an interaction between its non-signalling α-receptor, IL-6 receptor (IL-6R) and the signal transducing receptor, glycoprotein (gp) 130 [31, 32] (Fig. 1). Gp130 is ubiquitously expressed and due to its promiscuous receptor domain has an affinity for several cytokines. Conversely, IL-6-R specifically binds IL-6 and its expression is restricted to osteoclasts and osteoblasts [31]. The interaction of the two receptors forms a membrane-signalling complex, which specifically responds to IL-6. IL-6 first binds to IL-6R which then forms a heteromer with gp130 and stimulates the associated intracellular signalling machinery (JAK/STAT or MAPK pathways) and subsequent gene expression.

IL-6R exists as a membrane-bound form (mIL-6R) on the surface of osteoclasts and osteoblasts. However, the range of cells which can respond to IL-6 is expanded by an expression of the soluble form of the receptor (sIL-6R), which is able to interact with gp130 on cells that do not express mIL-6R, facilitating IL-6-mediated signalling in a process known as trans-signalling [33] (Fig. 1). In osteoporosis, the serum levels of sIL-6R are significantly increased and may be as important in the pathology of osteoporosis as levels of IL-6 itself [32, 34]. Indeed, variants of the IL-6R gene have been found to be associated with BMD in postmenopausal women [32].

IL-6, inflammatory diseases and osteoporosis

IL-6 has also been linked with the pathogenesis of osteoporosis in other chronic inflammatory conditions, such as inflammatory bowel disease (IBD) [35]. In a study of 104 patients with IBD, IL-6 levels were found to be elevated in patients with concurrent osteoporosis compared with non-osteoporotic patients. The role of IL-6 in osteoporosis has been further highlighted in a study of the expression of key regulatory molecules of bone remodel-ling in fragility fracture patients who needed hip surgery as a result of an intracapsular subcapital femoral neck fracture [36]. Expression of RANK and IL-6 were significantly elevated in the fracture group compared with an age-matched control group ($p<0.03$ and $p<0.002$, respectively). IL-6 mRNA levels associated strongly with RANKL mRNA levels ($r=0.77$, $p<0.001$) and RANK mRNA levels ($r=0.95$, $p<0.001$) in the fracture group, but not in the control group. These data suggest an association between IL-6 and RANKL/RANK and this is consistent with studies in murine osteoblastic cell lines, where IL-6 has been shown to induce RANKL mRNA expression [37]. It is thought that IL-6 utilises the RANK/RANKL/OPG interaction to exert an indirect effect on osteoclasts to promote osteoclast activation and therefore bone resorption [15]. This effect may occur via an interaction between IL-6 and osteoblasts, which may lead to increased osteoblastic RANKL production [15]. The effects of IL-6 and other cytokines on bone turnover are summarised in Fig. 2.

Other factors influencing IL-6 production and risk of osteoporosis

Menopausal status is a significant determinant of development of osteoporosis in patients with RA, and oestrogen has been shown to modulate IL-6 synthesis, such that IL-6 levels are increased in serum and in bone cells post-menopause and following ovariectomy [36, 38]. Furthermore, studies in IL-6-deficient mice suggest that they are able to maintain their bone mass after ovariectomy with no change in bone turnover, unlike wild-type animals [38]. In a mouse model of RA [39] raloxifene, a selective oestrogen receptor modulator, significantly decreased levels of IL-6 levels versus controls (73 versus 175 pg/ml, $p<0.01$) and also protected against both joint damage and osteoporosis as well as ameliorating both the severity and frequency of arthritis in these animals.
There have been relatively few studies investigating mediators of osteoporosis specifically in patients with RA. Sugiyama [40] investigated cytokine expression in postmenopausal women with RA and periaricular osteoporosis, compared with postmenopausal women with osteoarthritis (OA) and no bone loss. Reduced levels of circulating hormones, such as oestrogen, can lead to the activation of T cells and subsequent disruption of bone homeostasis by secretion of IL-6 and other cytokines, potentially stimulating systemic bone loss [41]. IL-6, TNF-α and IL-1β were all detected at significantly higher levels in the synovial fluid of the knee joints of patients with RA compared with patients with OA (p<0.001 for all; Table 1). Expression of IL-6 and IL-1β mRNA, but not TNF-α mRNA, was significantly higher in the knee joints of patients with RA compared with OA (p=0.005 and p=0.002, respectively; Table 1). Furthermore, expression of IL-6 mRNA, but not that of TNF-α and IL-1β, was significantly higher in the periarticular cancellous bone of the femoral condyle in patients with RA versus OA (p=0.024). IL-6 and prostaglandin E2 (PGE2) production in cells of the osteoblastic lineage was also significantly higher in RA patients (p<0.001). In vitro, IL-6 and PGE2 together have been shown to enhance osteoclastogenesis through effects on the RANK/RANKL/OPG system [42]. Inhibition of RANKL and subsequent osteoclast formation is a promising strategy for the treatment of osteoporosis, and potentially other disorders associated with bone loss. Cummings and colleagues recently described how treatment with the anti-RANKL antibody denosumab significantly reduced the risk of vertebral, nonvertebral and hip fractures in women who had osteoporosis compared with untreated subjects (p<0.05) [43].

Table 1 Levels and mRNA expression of TNF-α, IL-1β and IL-6 in the synovium of the knee joint in postmenopausal patients with rheumatoid arthritis and periaricular osteoporosis, and in postmenopausal patients with osteoarthritis [40]

<table>
<thead>
<tr>
<th></th>
<th>Patients with RA (n=8)</th>
<th>Patients with OA (n=15)</th>
<th>p value</th>
</tr>
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<tbody>
<tr>
<td>Levels in the synovial fluid of the knee joint, pg/ml</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>TNF-α</td>
<td>12.1±7.0</td>
<td>2.3±2.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IL-1β</td>
<td>9.27±7.64</td>
<td>0.73±0.65</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IL-6</td>
<td>7540±8450</td>
<td>156±201</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Relative mRNA expression in the synovium of the knee joint (versus β-actin)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNF-α</td>
<td>0.47±0.18</td>
<td>0.45±0.15</td>
<td>NS</td>
</tr>
<tr>
<td>IL-1β</td>
<td>1.06±0.23</td>
<td>0.55±0.30</td>
<td>0.002</td>
</tr>
<tr>
<td>IL-6</td>
<td>1.41±0.26</td>
<td>0.92±0.34</td>
<td>0.005</td>
</tr>
</tbody>
</table>

RA: rheumatoid arthritis, OA: osteoarthritis

Clearly there are many factors involved in the development of osteoporosis, and it is likely that concurrent RA further adds to this complexity. Given the scarcity of mechanistic studies of osteoporosis in patients with RA, further research is clearly warranted. The findings of such studies may help in the understanding of the role of inflammatory cytokines in this process and may also aid in the early identification of RA patients at highest risk of osteoporosis and in the selection of appropriate treatment.

Managing osteoporosis in patients with RA

Cross-sectional and longitudinal studies suggest that conventional treatments for RA, including disease-modifying antirheumatic drugs (DMARDs), such as methotrexate (MTX), do not have any adverse effects on BMD (reviewed in [44]). In contrast, it is well-known that treatment with corticosteroids results in loss of BMD. Indeed, a committee of the American College of Rheumatology (ACR) recognised the risk of glucocorticoid use and recommended screening for osteoporosis with BMD testing and subsequent treatment in patients with a T-score <-1.0 [45].

BMD is used to establish a diagnosis of osteoporosis and to monitor disease progression and response to treatment. However, fractures can occur in patients who do not have a T-score <-2.5 and changes in BMD in response to treatment can be slow (2–5%/year, or a maximum of <3% in 3–6 months) [46]. There has been recent interest in using biochemical markers of bone turnover for monitoring response to anti-cytokine treatment. Bone formation markers include: bone-specific alkaline phosphatase, which can be identified by chemical separation, automated assays have improved reproducibility between assays to <5%; osteocalcin is synthesised by osteoblasts, it can be measured using immuno-assays, however, degradation of osteocalcin can be a problem; procollagen I extension peptides are released from collagen when it is deposited into bone, concentration of the extension peptides in the serum can be assayed using immuno-methods and correlate with bone formation [46]. Bone resorption markers include: acid phosphatase which is osteoclasts, enzyme levels have used as a marker of bone resorption for some time; pyridinoline and deoxypyridinoline cross-links are formed between collagen molecules and are released into circulation when bone is catabolised; telopeptides of type I collagen are formed by the breakdown of collagen [46]. Relative levels of biochemical markers of bone turnover provide a useful tool for monitoring the response to anti-cytokine treatment.

Bisphosphonates are standard treatment for women with postmenopausal osteoporosis and, unless contraindicated, bisphosphonates are also recommended in ACR guidelines.
for management of osteoporosis in patients with RA. Etidronate, alendronate and risedronate have all been shown to increase BMD in patients with RA-associated bone loss [47–50], although the effect on fracture incidence has been limited. Similar results have been obtained with the anti-RANKL monoclonal antibody denosumab [51] indicating that the cytokine pathways regulating osteoclast differentiation and activation may be an important target for therapy of RA-associated osteoporosis. Interestingly, RANKL has recently been demonstrated to reduce bisphosphonate-induced apoptosis of osteoclasts [52].

While standard anti-osteoporosis treatments remain an important tool for managing bone loss in patients with RA, in recent years attention has focused on biological agents that target the underlying inflammatory processes that ultimately lead to loss of BMD in these patients. The anti-TNF-α antibody infliximab has demonstrated positive effects on BMD in a number of studies in patients with RA [20, 53, 54]. A case–control study was conducted to compare bone loss in 99 control RA patients who received MTX and 90 RA patients who received infliximab because of persistent active disease on MTX [54]. Lumbar and femoral neck BMD was maintained in patients treated with infliximab but significantly decreased in the control group (p<0.001). The protective effect of infliximab on BMD was not simply due to a reduction in RA disease activity: there was no correlation between BMD and clinical RA response in infliximab-treated patients, with even non-responders maintaining BMD.

Given the key role of IL-6 in RA and osteoporosis, and its interactions with TNF-α and RANK/RANKL, therapeutic blockade of the production and actions of IL-6 may offer potential strategies for the treatment of osteoporosis-associated RA. Indeed, as highlighted, raloxifene reduced IL-6 levels in an animal model of RA and protected against the development of osteoporosis [39].

Specific inhibition of IL-6R is an attractive option for treating RA. The humanised anti-IL-6R antibody, tocilizumab, has shown significant improvements in multiple measures of disease activity and quality of life in patients with RA in phase III trials [55, 56]. In addition, inflammatory markers such as C-reactive protein were significantly reduced by tocilizumab compared with placebo (p<0.001) [55, 56]. However, studies with anti-IL-6R antibodies have demonstrated an increase in lipid levels that coincide with the decrease in C-reactive protein levels [55, 56]. This may be a possible consequence of treating inflammation as patients with active rheumatoid arthritis have lower lipid concentrations than the general population. Increased lipid levels have been observed in RA patients treated with other agents such as TNF inhibitors, and are not associated with an increased risk of cardiovascular events [57]. Other observations of IL-6R blockade include changes to liver aminotransferase and neutrophil levels. However, these were not associated with clinical symptoms or increases in the occurrence of infections [55]. Importantly, no noticeable differences in efficacy and safety responses of background DMARD therapy were observed with concomitant treatment with tocilizumab [55]. Managing inflammation is likely to prove as a key to prevention of bone loss in patients with RA [58], and in this regard, blockade of IL-6 may represent a novel therapeutic approach for the treatment of both RA and osteoporosis.

Conclusions

Osteoporosis and associated fractures are common in patients with RA and result in major disability, reduced quality of life and increased healthcare costs. The social and economic consequences can only be prevented by effective treatments. Many processes contribute towards the pathology of RA-associated osteoporosis; however, increased osteoclast activation and subsequent bone resorption, mediated by IL-6, other inflammatory cytokines and disturbances in the RANK/RANKL/OPG system, are thought to play a key role. Targeted agents that specifically address underlying perturbations in RA-associated osteoporosis may offer an alternative to more general osteoporosis treatments.

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