

Review

A roadmap to target interleukin-6 in osteoarthritis

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Abstract

Joint inflammation is present in the majority of OA patients and pro-inflammatory mediators, such as IL-6, are actively involved in disease progression. Increased levels of IL-6 in serum or synovial fluid from OA patients correlate with disease incidence and severity, with IL-6 playing a pivotal role in the development of cartilage pathology, e.g. via induction of matrix-degrading enzymes. However, IL-6 also increases expression of anti-catabolic factors, suggesting a protective role. Until now, this dual role of IL-6 is incompletely understood and may be caused by differential effects of IL-6 classic vs trans-signalling. Here, we review current evidence regarding the role of IL-6 classic- and trans-signalling in local joint pathology of cartilage, synovium and bone. Furthermore, we discuss targeting of IL-6 in experimental OA models and provide future perspective for OA treatment by evaluating currently available IL-6 targeting strategies.

Key words: interleukin-6, osteoarthritis, IL-6 trans-signalling, therapy, cartilage, synovitis

Introduction

OA is a degenerative joint disease with increasing incidence due to a rise in life expectancy and average body weight in western society [1, 2]. Currently, therapies are focused on pain management or eventually joint replacement. OA affects all joint tissues, resulting in loss of articular cartilage, ectopic bone formation, subchondral bone sclerosis and synovial inflammation [3]. Inflammation is increasingly accepted as a driver of OA pathology, implying the synovium and inflammatory cytokines in driving cartilage degeneration [4–6]. For this reason, treatment strategies have focused on targeting pro-inflammatory cytokines TNF- α and IL-1 β in hand and knee OA [7–10], which did not result in clinical applications thus far. Therapies targeting IL-6 are approved and effective in treating RA, juvenile idiopathic arthritis, Castleman's disease, and giant cell arteritis [11]. Also in OA, IL-6 plays a significant role in joint pathology, but has not been a primary target of interest as research mostly focused on IL-1 β and TNF- α . Here, we review the current state of evidence regarding the role of IL-6 in OA pathophysiology, and discuss potential therapeutic approaches to target the IL-6 signalling pathway in OA.

Understanding the complexity of the IL-6 signalling pathway

Intracellular signalling cascades

IL-6 signalling starts by binding of IL-6 to the IL-6 receptor α subunit (IL-6R), followed by complex formation with a homodimer of glycoprotein 130 (gp130) [12]. The IL-6R has no signal transduction capacity and its expression is limited, e.g. to monocytes, hepatocytes and certain leucocyte subsets [13]. In contrast, the signal-transducing receptor gp130 is ubiquitously expressed. Gp130 also functions as a β subunit for other IL-6 family cytokines, like oncostatin-M, IL-11, IL-27 and leukemia inhibitory factor [14]. After IL-6 receptor complex formation, the Janus kinases/signal transducers and activators of transcription (JAK/STAT) pathway is activated (Fig. 1), leading to recruitment and activation of STAT1, STAT3, and to a lesser extent STAT5 [15]. Besides canonical signalling via JAK/STAT, IL-6 activates non-canonical signalling via mitogen-activated protein kinase (MAPK) cascade (Ras-Raf-MEK-ERK pathway) and PI3K- protein kinase B (Pkb)/Akt. IL-6-induced JAK/STAT is tightly controlled by negative feedback regulators, such as suppressor of cytokine signalling (SOCS) protein family and protein inhibitors of activated STATs (PIAS) [16, 17]. SOCS proteins are directly induced by gp130 cytokines, resulting in a negative feedback loop. SOCS3 has been identified as a specific inhibitor of IL-6 signalling and directly inhibits JAK-kinase activity [18, 19]. PIAS negative inhibitors are constitutively expressed and inhibit DNA-binding activity by binding to activated STAT-dimers.

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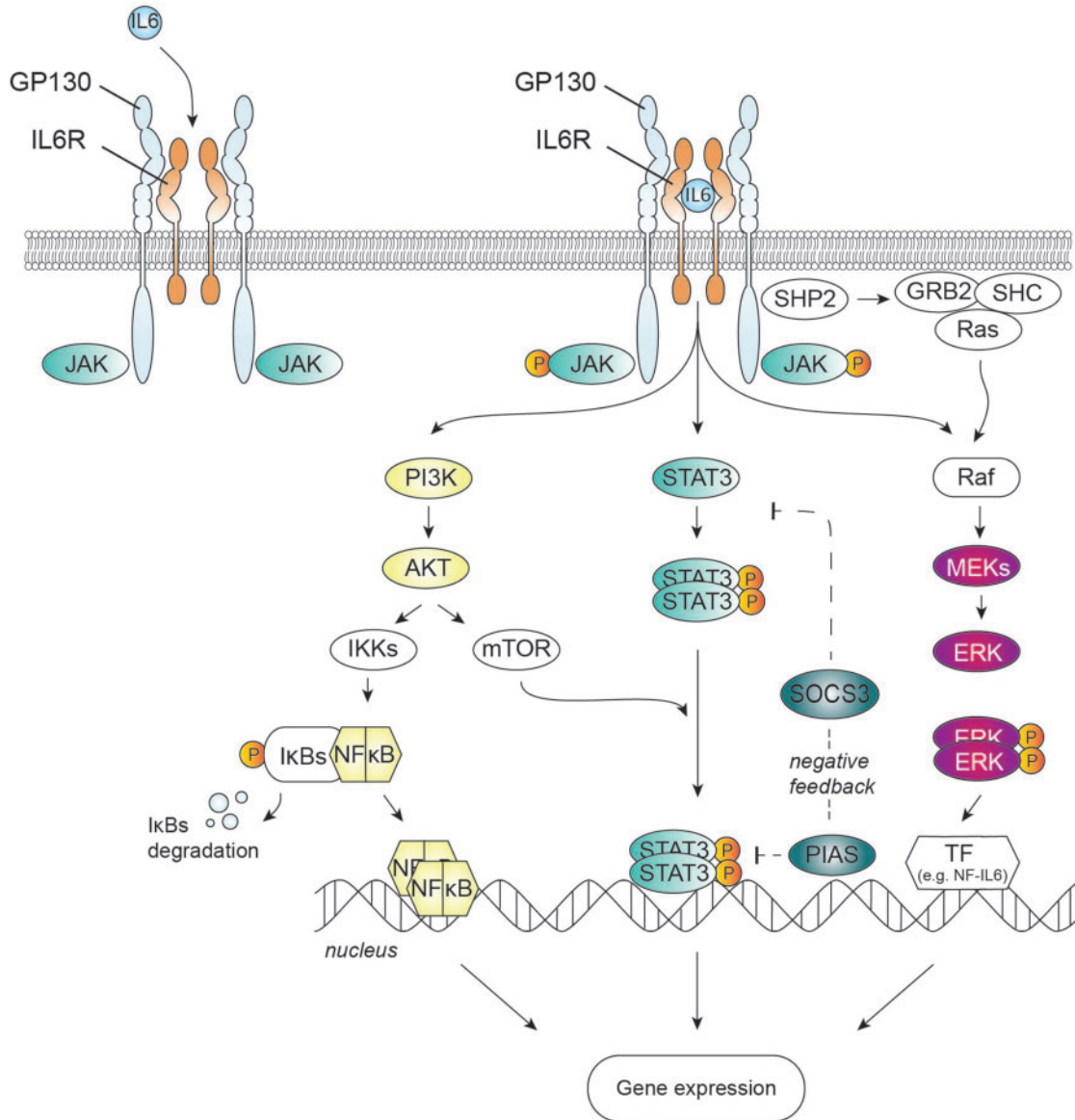
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Rheumatology key messages

- IL-6 signalling is actively involved in OA pathology, identifying IL-6 as a promising therapeutic target.
- Differences between classic- vs trans-signalling explain the protective and degenerative IL-6 effects in joint tissues.
- Specific targeting of IL-6 trans-signalling could be a superior treatment strategy in OA.

Fig. 1 Overview of IL-6 signalling pathways



After IL-6 binding to the IL-6R, complex formation with gp130 initiates phosphorylation of JAKs resulting in activation of STAT3-, PI3K- and Ras-Raf-MEK-ERK signaling. Activated transcription factors (e.g. STAT3, NF- κ B and NF-IL-6) translocate to the nucleus to regulate target gene expression. SOCS and PIAS proteins negatively regulate IL-6-induced JAK-STAT signal by blocking JAK-mediated activation of STAT3 (SOCS3), or by blocking DNA-binding activity of STAT3 (PIAS). gp130: glycoprotein 130; IL-6: interleukin-6; JAK: janus kinase; MAPK: mitogen-activated protein kinase; NF- κ B: nuclear factor kappa-light-chain-enhancer of activated B cells; NF-IL6: a nuclear factor for IL-6 expression; PIAS: protein inhibitors of activated STATs; PI3K: phosphoinositide 3-kinase; SOCS3: suppressor of cytokine signaling 3; STAT3: signal transducer and activator of transcription 3.

Cytokine interplay and intracellular cross-talk

Interplay between IL-6 signalling pathways and other cytokines exists on multiple levels [14]. For example, other cytokines from the IL-6 family, like ciliary neurotrophic factor (CNTF) and IL-30, can also bind and activate the IL-6R, although with lower binding affinity compared with the CNTF- and IL-30 receptors [20, 21]. Furthermore, interplay between IL-6 and pro-inflammatory cytokine signalling may restrict uncontrolled pro-inflammatory signalling [22]. For instance, IL-1 β strongly inhibits IL-6-mediated acute phase reaction in the liver by directly inhibiting p38 MAPK-dependent STAT3 phosphorylation [22, 23]. More specifically, MAPK p38 and the transcription factor NF- κ B were identified as crucial regulators of the IL-6 signalling pathway [22]. Also, interplay between IL-6 and anti-inflammatory cytokines, such as TGF- β , is present at receptor level and at the level of intracellular mediators [24–26]. Crosstalk between STAT3 and Smad3, the main intracellular mediator of TGF- β signalling, exists in diverse pathophysiological conditions and leads to either synergistic or antagonistic actions depending on cell type and context [26].

Modes of IL-6 signalling

IL-6 has the unique ability to initiate signal transduction via different modes of receptor activation. Signalling via membrane-anchored IL-6R (mIL-6R) is termed classic signalling and is important for the acute-phase response, hematopoiesis and central homeostatic processes [27] (Fig. 2a). Interestingly, a soluble form of IL-6R (sIL-6R) can be produced by shedding of membrane-bound receptor or alternative splicing [28, 29]. sIL-6R can bind secreted IL-6, forming a complex that increases the half-life of IL-6 [30]. Signalling via sIL6R is called trans-signalling and greatly broadens the scope of IL-6 responsiveness, as any gp130-expressing cell can bind and respond to the IL-6/sIL-6R complex (Fig. 2b). IL-6 trans-signalling mainly regulates pro-inflammatory events and is implicated in numerous chronic diseases and cancers [27, 31]. Trans-signalling leads to stronger activation of IL-6 intracellular signalling routes, resulting in enhanced target gene expression, but how this works is still unclear [32, 33]. Possibly, restricted expression of mIL-6R limits activation of STAT3 via classic signalling, but not trans-signalling due to additional presence of sIL-6R [32]. Within our circulation, a soluble form of gp130 (sgp130) acts as a natural inhibitor of IL-6 trans-signalling by binding to the IL-6/sIL-6R with high affinity [31, 34–36]. Sgp130 therefore specifically inhibits IL-6 trans-signalling and does not affect classic signalling or recently discovered IL-6 cluster-signalling. IL-6 cluster-signalling involves membrane IL-6/IL-6R complexes on dendritic cells, which activate gp130 receptors on receiving T cells resulting in the generation of pathogenic Th17 cells (Fig. 2c). [37]. Whether IL-6 cluster-signalling is also relevant in other biological settings remains to be investigated.

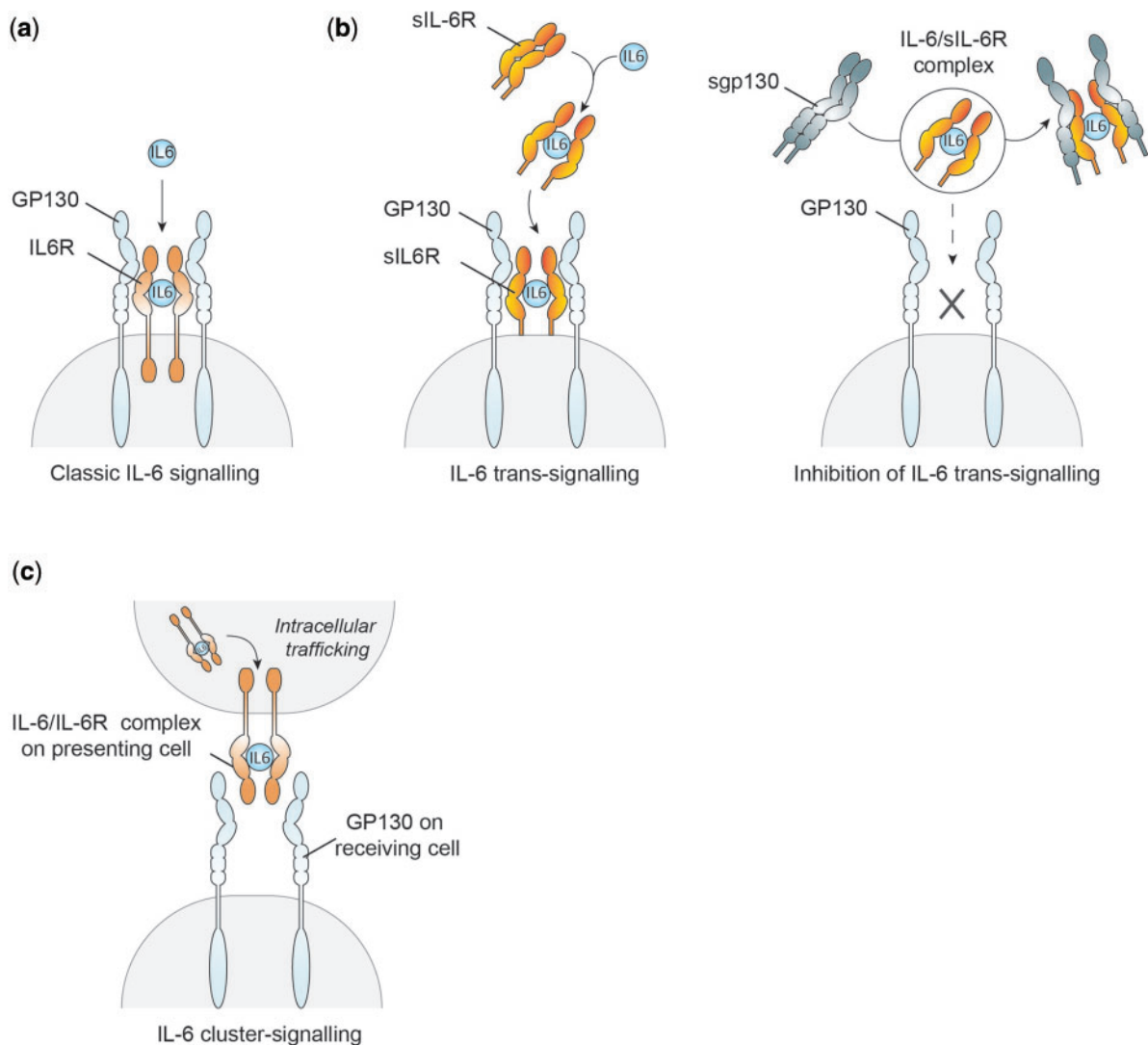
Local and systemic perspective: levels of IL-6 and its soluble receptors in OA

IL-6 levels relate to OA incidence and pathology

IL-6 was detected in OA synovial fluid (SF) as early as 1988. However, this did not result in follow-up studies, as IL-6 levels were lower compared with RA SF and healthy controls were not included [38, 39]. Later, it became clear that IL-6 levels were significantly increased in OA SF and serum compared with healthy individuals [40–42]. Furthermore, additional studies showed a clear relation between systemic IL-6 levels and OA incidence [43–45]. Increased circulating levels of IL-6 were predictive for knee OA and cartilage loss in 3 and 15 years in two independent follow-up studies [43, 44]. Moreover, a high innate capacity to produce IL-6, in response to lipopolysaccharide stimulation, was associated with hand OA development in 90-year-old individuals [45]. Higher IL-6 levels in OA serum or SF also correlate with disease progression or severity of cartilage pathology [42, 46–48]. This suggests that IL-6 levels may reflect cartilage damage, which is supported by the fact that SF IL-6 levels are strongly increased in individuals with cartilage defects but no macroscopic signs of OA [49, 50]. When local vs systemic levels of IL-6 were compared in the same patients, IL-6 concentrations were higher in OA SF (119.8 ± 193.5 pg/ml) compared with plasma samples (3.1 ± 2.7 pg/ml) [51]. Furthermore, two patient subgroups can be identified based on IL-6 levels in OA SF, high producers (2022 ± 526 pg/ml) vs average producers (132 ± 19 pg/ml), of which high producers may particularly benefit from IL-6 targeted therapy [52].

Local production of IL-6 by joint tissues

It is now recognized that synovial inflammation is important in OA, and synovitis is observed in ~50% of OA patients [5, 53]. The synovium is an important producer of IL-6 in OA, for instance via (activated) synovial fibroblasts or plasma cells in the synovial lining [52, 54–56]. Besides the synovium, the infrapatellar fat pad (IFP) is an important source of IL-6. The IFP is the main fat tissue within the knee, and actively contributes to OA pathophysiology via production of pro-inflammatory mediators and adipokines [57]. Interestingly, the IFP from knee OA patients secreted significantly higher levels of IL-6, but not TNF- α and IL-1 β , compared with abdominal fat tissue from the same patients [58]. Furthermore, IFP-conditioned medium cultured with traumatized cartilage explants caused IL-6-dependent glycosaminoglycan release [59]. Also, synovial fibroblasts from obese OA patients secreted higher levels of IL-6 compared with normal-weight patients [60], indicating that IL-6 may be particularly relevant in obesity-derived OA, especially as IL-6 plays a central role in cell metabolism [61].

Fig. 2 Modes of IL-6 signalling

(a) Classic IL-6 signalling involves cells expressing both membrane (m)IL-6R and gp130; free IL-6 binds to mIL-6R, forming a complex with gp130. **(b)** IL-6 trans-signalling is activated by pre-formed complexes of IL-6 and soluble IL-6R (IL-6/sIL-6R) and requires only gp130 expression on target cells. Soluble gp130 (sgp130) acts as a natural inhibitor of trans-signaling by specifically binding to IL-6/sIL-6R complexes. **(c)** IL-6 cluster signaling utilizes gp130 on receiving cells, activated by IL-6/mIL-6R complexes on presenting cells (e.g. dendritic cells). gp130: glycoprotein 130; IL-6: interleukin-6; IL-6R: IL-6 receptor.

Broadening our horizon: levels of soluble IL-6 receptors in OA

The ratio of IL-6 classic- vs trans-signalling is regulated by sIL-6 receptors [62]. Increased levels of sIL-6R in OA patients may direct future treatment towards specific inhibition of IL-6 trans-signalling, while decreased sgp130 levels could indicate reduced negative feedback capacity. Unfortunately, studies investigating soluble IL-6 receptors in OA are scarce. Systemically, no differences were detected in sIL-6R levels in serum of healthy donors and OA patients [63], and as far as we know there is no study investigating systemic changes in

sgp130 in OA. In OA SF, both sIL-6R and sgp130 are present, but a comparison to healthy individuals is lacking [64–66]. Despite high levels of IL-6 production, it remains unclear if the synovium is a source of sIL-6 receptors in OA. In RA-derived material, cultured SF mononuclear cells produced sIL-6R, but not cultured chondrocytes or synovial cells [64]. However, this was not confirmed in OA. A potential source of sIL-6R in OA could be the IFP, which was shown to produce both IL-6 and sIL-6R [58], possibly resulting in IL-6 trans-signalling.

Direct effects of IL-6 on local joint tissues

Cartilage

As cartilage is the main OA affected tissue, previous studies mostly focused on identifying IL-6 effects in cartilage. However, IL-6 has both catabolic and protective effects in cartilage, which is still not completely understood. Early studies focused on IL-6 classic signalling and generally found protective effects of IL-6, such as production of tissue inhibitor of metalloproteinases (TIMPs) [67, 68]. Furthermore, IL-6 classic signalling slightly stimulated proteoglycan synthesis in human OA chondrocytes [49], and did not affect proteoglycan synthesis of human or bovine chondrocytes [69, 70]. However, detrimental effects of IL-6 classic signalling in cartilage have also been reported. IL-6 inhibited proteoglycan synthesis in human cartilage explants and rabbit chondrocytes [71–73]. Moreover, IL-6 suppressed collagen type II neo-synthesis and enhanced IL-1 β -mediated proteoglycan degeneration in rabbit chondrocytes [73, 74]. Several studies show that IL-6 induces metalloproteinase (MMP)-3, MMP-13 and A Disintegrin and Metalloproteinase with Thrombospondin motifs (ADAMTS) enzyme expression, which mediate cartilage degeneration [75–77]. Besides regulating matrix synthesis and degeneration, IL-6 induces matrix mineralization via formation of basic calcium phosphate crystals leading to proteoglycan loss [78]. Furthermore, IL-6 disturbs several other chondrocyte functions, resulting in decreased proliferation or increased oxidative stress generation [73, 79]. Of note, IL-6 directly induces SOCS3 expression, which can lead to insulin-like growth factor 1 desensitization in cartilage [80]. However, enhanced SOCS3 may also be protective as it restricts pro-inflammatory signalling in chondrocytes [81]. Altogether, this indicates that the definition of IL-6 classic signalling as only ‘protective’ is probably too simplified.

Generally, chondrocytes are considered to have low levels of mIL-6R, which may limit STAT3 activation, but strong evidence for this is missing [32, 82]. In murine epiphyseal chondrocytes, no mIL-6R expression was observed with flow cytometry [82]. In contrast, expression of mIL-6R was observed in four donors of OA human chondrocytes on both mRNA and protein level [25]. Chondrocyte sensitivity for classic IL-6 signalling is determined by mIL-6R levels, which can be altered by hormones, cytokines and epigenetic factors [13]. The cytokine IL-1 β increases mIL-6R expression in chondrocytes and hepatocytes [25, 83], which may explain synergistic effects of IL-1 β and IL-6 in mediating cartilage degradation or collagen breakdown [71, 73, 84]. Cartilage injury induced by blunt trauma also potentiated IL-6-mediated expression of catabolic markers in bovine cartilage, but the underlying mechanism was not investigated [59, 85]. On the other hand, TGF- β decreases mIL-6R expression in chondrocytes, resulting in inhibition of classic IL-6 signalling [25]. Also, mechanical compression of cartilage, which leads to active TGF- β

signalling [86], inhibited catabolic effects of IL-6 and TNF- α combined [87]. Changes in mIL-6R expression in chondrocytes alter their sensitivity towards IL-6 classic signalling and may partly explain previous discrepancies regarding IL-6 effects in cartilage.

With respect to IL-6 trans-signalling in cartilage, functional studies also show contradictory results. Most studies conclude that IL-6 trans-signalling is detrimental for cartilage, as it inhibits proteoglycan synthesis and stimulates proteoglycan loss [72, 88]. Moreover, sIL-6R was required for full activation of JAK1/2 in bovine chondrocytes, resulting in decreased expression of matrix components and increased levels of cartilage degrading enzymes [89, 90]. On the other hand, soluble IL-6R also augmented production of anti-catabolic TIMPs in chondrocytes [67], which suggests that sIL-6R stimulates general IL-6 signalling and not only the catabolic response. In conclusion, there is substantial evidence for a direct role of IL-6 in regulating chondrocyte function and cartilage metabolism. While most studies report catabolic effects of IL-6 on cartilage, protective effects are also found. These discrepancies may be explained by functional differences in IL-6 classic vs trans-signalling, or disturbed expression of mIL-6R levels on chondrocytes.

Other joint tissues

As OA is a whole joint disease, direct effects of IL-6 on other joint tissues such as synovium, subchondral bone, and muscle tissue are also of interest [3]. Comparable to IL-6 effects in cartilage, mainly IL-6 trans-signalling was associated with detrimental effects in synovium. In OA- or RA-digested synovium, both IL-6 classic and trans-signalling increase production of TIMP, but only trans-signalling induces expression of ADAMTS4 [67, 68, 91, 92]. Moreover, IL-6 trans- but not classic signalling caused strong proliferation of RA synovial fibroblasts that could indicate a role in synovial hyperplasia or fibrosis [93]. However, in bone this distinction is less clear. On the one hand, IL-6 trans-signalling promotes osteoclast formation and consequently bone resorption, while classic signalling inhibits osteoclastogenesis [39, 94, 95]. On the other hand, IL-6 trans-signalling has been shown to promote bone formation [96]. The dual effect of IL-6 trans-signalling on bone resorption and formation may be explained by variation in levels of the pro-osteoclastogenic factor receptor activator of nuclear factor kappa-B ligand (RANKL). While high-levels of RANKL promote osteoclastogenesis, lower RANKL levels result in inhibition of osteoclast formation [97]. Finally, IL-6 may be directly involved in the process of OA-related muscle degeneration [98, 99]. Indeed, elevated levels of IL-6 are associated with reduced muscle endurance in elderly women with knee OA [100]. Moreover, increased levels of IL-6, STAT3 and SOCS3 have been detected in muscle tissue of knee OA patients [101]. However, IL-6 also mediates important anabolic processes in muscle tissue, such as muscle growth and myogenic differentiation [102]. This dual role

of IL-6 in muscle tissue might be a result of functional differences in IL-6 classic- vs trans-signalling, but this is yet unknown. Thus, IL-6 classic and trans-signalling can affect joint tissues besides articular cartilage, but their respective functional effects and role in OA development remain elusive.

Evidence for a role of IL-6 in OA pathology: lessons learned from animal studies

The role of IL-6 in OA pathophysiology has been studied in several experimental OA models and mostly show a destructive role for IL-6 (Table 1). Induction of the destabilization of the medial meniscus (DMM) model in IL-6^{-/-} mice resulted in marked reduction of cartilage destruction compared to wildtype mice [75]. Moreover, expression levels of MMP-3 and MMP-13 were significantly decreased in IL-6^{-/-} mice compared to wildtype, indicating that IL-6 induces catabolic mediators in OA. This catabolic role of IL-6 was supported by overexpression of HIF-2 α in wildtype vs IL-6^{-/-} mice, causing OA-like cartilage destruction in wildtype but not IL-6^{-/-} mice. Local injection of IL-6 into the knee joint caused significantly increased cartilage degeneration and MMP-3 and -13 expression, revealing direct evidence for deleterious effects of IL-6 in OA [75]. Strikingly, despite marked evidence of IL-6 involvement in OA, only one study blocked IL-6 itself. Both systemic administration of a neutralizing antibody as well as anti-IL-6 siRNA resulted in decreased cartilage lesions and subchondral bone sclerosis in the anterior cruciate ligament transection (ACLT) OA model [103]. Moreover, systemic treatment with an anti-IL-6-receptor neutralizing antibody (MR16) in the DMM model ameliorated the extent of cartilage pathology, synovial inflammation and osteophyte development [105]. This antibody is similar to tocilizumab, which directly targets the human IL-6R and is clinically effective in several inflammatory diseases [11]. Moreover, blocking of the IL-6R using tocilizumab resulted in cartilage preservation in a mouse model of ischemic osteonecrosis and significantly increased bone volume [106].

IL-6 is possibly mainly involved in trauma-related OA, as both the DMM and ACLT models reflect trauma-induced OA development. Also, in humans, local IL-6 levels strongly increase upon cartilage trauma and associate with knee OA progression after previous meniscectomy [49, 50]. Systemic IL-6 levels also increase during natural ageing and are associated with several age-related diseases [107, 108]. However, there was no difference in cartilage degradation in wildtype or IL-6^{-/-} mice after age-related OA development [109]. Male IL-6^{-/-} mice even developed more cartilage damage, ectopic bone formation and subchondral bone sclerosis compared to male wildtype mice, while there was no difference in pathology of females. This suggests that IL-6 has no pathological role in age-related murine OA, and even ameliorates OA pathology in male mice.

Multiple studies show interplay between IL-6 and sex hormones such as testosterone and oestrogen [110–112], but it is unclear how sex hormones affect IL-6 function within the joint. Of note, no markable inflammation was detected in these mice including inflammatory infiltrate, exudate or synovitis [109]. This raises the question whether murine ageing fully reflects human age-related OA development, in which synovitis is commonly observed and levels of inflammatory mediators, amongst which IL-6, are systemically increased [5, 53, 107]. Besides age-related OA, there was no difference in OA pathology caused by collagenase-induced OA (CiOA) between wildtype or IL-6^{-/-} mice [109]. Although using conditional IL-6^{-/-} mice, instead of constitutive knockouts, would more closely resemble physiological conditions, this suggests that other mediators may cause OA pathology in this model. However, to really exclude a role for IL-6 in CiOA and age-related OA, lack of IL-6 effects should be confirmed by independent studies. Functional differences in IL-6 classic vs trans-signalling may explain the contradictory results obtained in the different OA models. Unfortunately, all of the employed blocking strategies block both the classic- and trans-signalling pathway and current studies do not report sIL-6R levels. Specific inhibition of IL-6 trans-signalling in OA models might be extremely helpful to dissect detrimental vs protective effects of IL-6 in the future.

Blocking downstream of IL-6: targeting STAT3 in experimental OA

STAT3 is the most specific downstream mediator of the IL-6 signalling pathway, but is not solely activated by IL-6. Therefore, STAT3 activation in OA results from synergistic actions of several gp130 cytokines [14]. Hypothetically, targeting of STAT3 may be more successful compared with blocking IL-6, as other STAT3-activating cytokines also have catabolic and inflammatory effects on cartilage [113]. Indeed, repeated administration of a small molecule inhibitor against STAT3 (Stattic) in the DMM model resulted in stronger protection against cartilage degeneration and osteophyte formation compared with blocking IL-6R [105]. This additional effect may result from blockade of both IL-6 and oncostatin M (OSM) signalling via STAT3, based on the role of OSM in osteophyte proliferation and synovial inflammation [104, 114]. Inhibition of JAK2/STAT3 signalling in the ACLT model using the AG490 inhibitor also led to considerable protection against cartilage degeneration and subchondral bone sclerosis [103]. However, mesoderm-specific deletion of STAT3 caused expansion of growth plate hypertrophic chondrocytes and severe dysregulation of endochondral ossification, caused by STAT3-mediated activation of Sox9 in chondrocytes [115]. This phenotype is not observed in IL-6^{-/-} animals [116], suggesting that other STAT3-activating cytokines may cause dysregulation of cartilage and bone development, such as LIF which is associated with reduced skeletal growth [117]. Recently, a novel

TABLE 1 Effect of targeting IL-6 signalling in experimental OA models

IL-6 targeting strategy	Animal (sex, strain, age)	OA model	Observations	Effect	References
General knock-out (IL-6 ^{-/-})	Male C57BL/6 mice (10–12 weeks)	Surgically-induced post-traumatic OA (DMM)	↓ cartilage pathology	+	[75]
Systemic treatment with anti-IL-6 antibody	Male C57BL/6 mice (8 weeks)	Surgically-induced post-traumatic OA (ACLT)	↓ cartilage pathology	+	[98]
Systemic treatment with STAT3 inhibitor(AG490)	Male C57BL/6 mice (8 weeks)	Surgically-induced post-traumatic OA (ACLT)	↓ cartilage pathology ↓ subchondral bone sclerosis	+	[98]
Systemic treatment with anti-IL-6R antibody(MR16-1)	Male C57BL/6 mice (10 weeks)	Surgically-induced post-traumatic OA (DMM)	↓ cartilage pathology ↓ osteophyte formation ↓ synovitis	+	[99]
Systemic treatment with STAT3 inhibitor(Stattic)	Male C57BL/6 mice (10 weeks)	Surgically-induced post-traumatic OA (DMM)	↓ cartilage pathology ↓ osteophyte formation	+	[99]
General knock-out (IL-6 ^{-/-})	Male C57BL/6 mice (18–23 months)	Age-related OA (Spontaneous OA)	↑ cartilage loss ↑ subchondral bone sclerosis	-	[103]
General knock-out (IL-6 ^{-/-})	Female C57BL/6 mice (18–23 months)	Age-related OA (Spontaneous OA)	No difference	No effect	[103]
General knock-out (IL-6 ^{-/-})	Male and female C57BL/6 mice (3–4 months)	Chemically-induced joint instability OA (CiOA)	No difference	No effect	[103]
Intra-articular treatment with gp130 modulator (RCGD 423)	Male Sprague-Dawley rats (3–4 months)	Surgically-induced post-traumatic OA (Partial meniscectomy)	↓ cartilage pathology ↓ osteophyte formation ↓ chondrocyte proliferation	+	[104]

ACTL: anterior cruciate ligament transection; CiOA: collagenase-induced OA; DMM: destabilization of the medial meniscus.

gp130-small molecule modulator (RCGD 423) was discovered, which directed gp130 towards proliferative STAT3/c-Myc signalling, while inhibiting ERK/NF- κ B signalling. Therapeutic administration of the RCGD 423 compound, leading to STAT3 activation, resulted in reduced cartilage degeneration in a rat partial meniscectomy model [118]. This contradicts the earlier finding that STAT3 inhibition using Stattic protects against cartilage degeneration [105]. It is possible that the proliferative effect of the RCGD423 inhibitor is caused by the strong activation of c-Myc, as LIF-driven c-Myc activation is critical for chondrocyte survival and proliferation in fetal cartilage [118]. These opposing results indicate that the ultimate result of STAT3 inhibition, beneficial or detrimental, is strongly context-dependent and determined by the integrated signal of multiple gp130 cytokines. While targeting of STAT3 may seem promising in experimental OA, this might prove difficult in OA patients due to large differences in severity and incidence of inflammation, and heterogeneity in STAT3-activating cytokines or growth factors [53]. This makes it difficult to predict the outcome of STAT3 inhibition in OA and argues for the simpler approach of directly targeting gp130 cytokines, such as IL-6, upstream of STAT3.

Under construction: IL-6 targeted therapy in OA

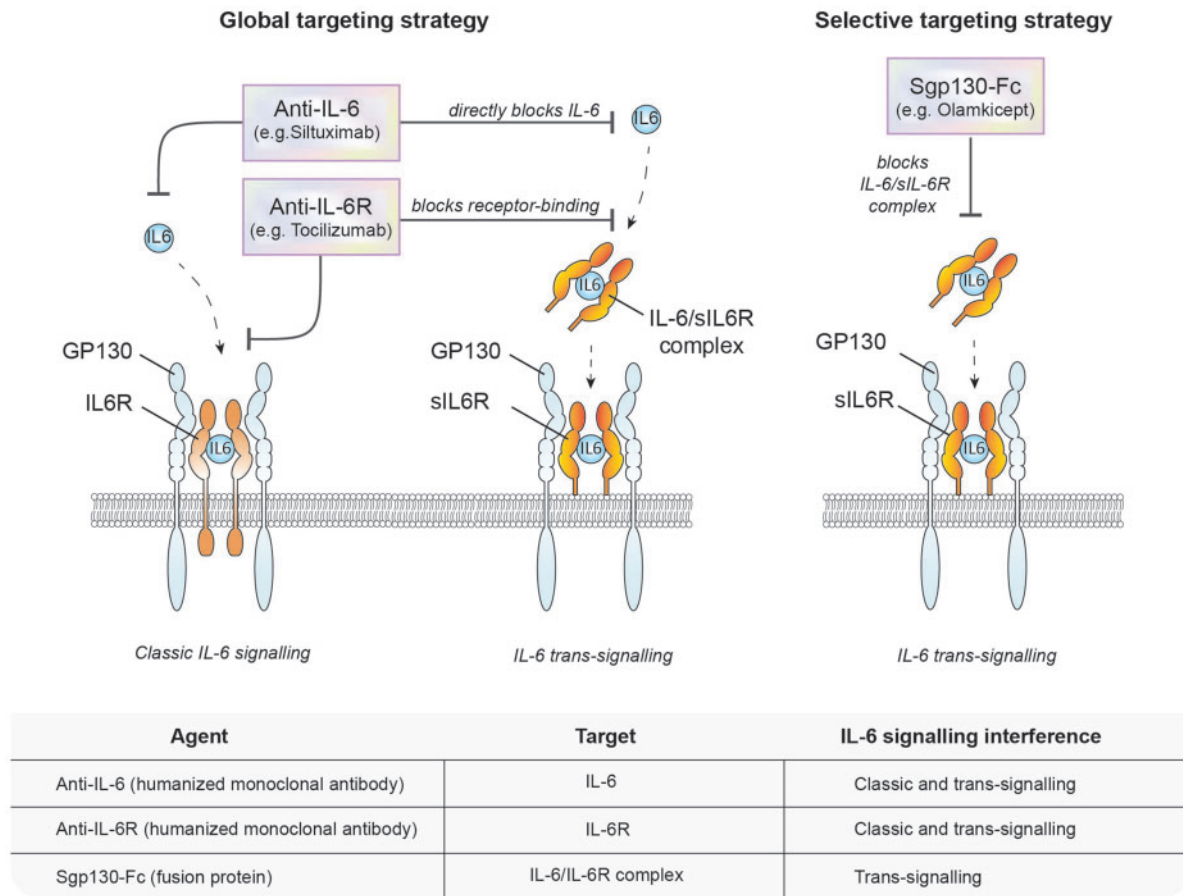
Currently, multiple therapeutic strategies exist to effectively target the IL-6 signalling pathway and are safely applied for the treatment of several inflammatory diseases. For example, the IL-6R targeting antibody tocilizumab has been approved for treatment of RA, juvenile idiopathic arthritis, Castleman's disease and recently also for giant cell arteritis [62, 119]. Currently, no therapies targeting IL-6 signalling are approved for treatment of OA. This may change in the near future, as tocilizumab is being tested in a phase 3 randomized controlled trial in patients with refractory hand OA (ClinicalTrials.gov NCT02477059).

Previous studies showed that levels of IL-6 in SF can vary between different joints, which could direct future IL-6 targeted therapy towards relevant patient subgroups. For example, levels of IL-6 were strikingly higher in knee OA SF compared with carpometacarpal joint fluid [120]. Moreover, more IL-6 was detected in post-traumatic wrist OA compared with knee OA patients [121]. As inflammation is strongly linked to structural damage in hand OA patients [122–124], this patient group may be very suitable to study the consequences of blocking pro-inflammatory cytokines, such as TNF- α [125] and IL-1 β [7] and now IL-6. Besides stratification of patients based on joint location, treatment choice could also be based on OA subtypes. Post-traumatic OA is a common form of OA, developing after joint injury (e.g. anterior cruciate ligament or meniscus injury) [126]. In these patients, there may be a therapeutic window after injury, in which targeting of

inflammatory mediators may prevent the development of further damage. During joint injury, such as anterior cruciate ligament rupture, levels of IL-6 in SF are highly increased up to 1000-fold [127, 128], and a sudden increase in IL-6 levels has also been found after focal cartilage damage [49, 50]. This suggests that inhibition of IL-6 shortly after joint injury may be a promising treatment strategy to prevent the development of post-traumatic OA; however, the optimal therapeutic window to prevent further damage is still unknown.

Due to the success of tocilizumab, novel IL-6 pathway inhibitors have been developed, such as biologics targeting IL-6R (vobarilizumab, satralizumab, sarilumab), IL-6 (siltuximab, olokizumab, sirukumab, clazakizumab and MEDI 5117), IL-6 trans-signalling (olamkicept), or small molecule inhibitors directed against JAKs or STAT3 [129]. Multiple inhibitors have been developed and clinically tested that target JAK-kinases or STAT3 directly [11]. Although some of these compounds are clinically effective in RA, such as tofacitinib and baricitinib (pan-JAK inhibitors), they have not been tested in OA patients [130]. Yet, there are pre-clinical indications that JAK/STAT inhibition could be effective in OA. For instance, tofacitinib inhibited cytokine-induced proteoglycan loss and restored collagen type II synthesis in bovine cartilage explants [131]. In addition, animal studies indicate protective effects of JAK/STAT inhibition in experimental OA [103, 105]. However, targeting of JAK/STAT signalling also results in inhibition of multiple cytokines including IL-10, IL-4 and IGF-1, which have a beneficial role in joint biology and OA development [14, 132, 133]. As OA is a very heterogeneous disease with large differences in severity of inflammation and cytokine profile [53], it will be difficult to predict outcome of JAK/STAT inhibition in OA patients. Therefore, the simpler approach of targeting one cytokine, like IL-6, might be a safer strategy. As several therapeutics have been developed that target IL-6 signalling via a different mechanism, the comparison of these treatments will greatly enhance knowledge about the role of IL-6 in disease. Tocilizumab, for instance, blocks all IL-6 signalling pathways (classic and trans-signalling, and potentially also cluster-signalling [37]), because it inhibits IL-6 binding to both mIL-6R and sIL-6R (Fig. 3) [134]. In contrast, olamkicept specifically targets the IL-6/sIL-6R complex, thereby only inhibiting IL-6 trans-signalling and not classic signalling. Olamkicept is a fusion protein consisting of two soluble human gp130 proteins fused with the Fc region of human IgG (sgp130Fc) [129]. Accordingly, olamkicept blocks pro-inflammatory events of IL-6 trans-signalling, while simultaneously allowing homeostatic effects of IL-6 classic signalling. Olamkicept was already successfully used in treating experimental Crohn's disease and is now in clinical trials for inflammatory bowel disease and active ulcerative colitis [11, 135]. Specific inhibition of IL-6 trans-signalling could be a preferred future treatment strategy, as several side effects of tocilizumab have already been reported that may result from inhibition of IL-6 classic signalling, such

Fig. 3 Current IL-6 targeting strategies



Anti-IL-6 monoclonal antibodies (e.g. siltuximab) directly target IL-6, thus blocking both classic and IL-6 trans-signaling. IL-6R targeting antibodies (e.g. tocilizumab) block binding of IL-6 to the IL-6R (both mIL-6R as well as sIL-6R), thereby inhibiting IL-6 classic and trans-signaling pathways. The sgp130Fc fusion protein (e.g. olamkicept) was developed to specifically target IL-6 trans-signaling, and only binds to the IL-6/sIL-6R complex. Sgp130Fc does not bind to membrane IL-6R or free IL-6, therefore allowing classic IL-6 signaling to continue. IL-6: interleukin 6; mIL-6R: membrane IL-6 receptor; sgp130: soluble glycoprotein 130; sIL-6R: soluble IL-6 receptor.

as impairment of the acute phase response [129]. Olamkicept might also be a promising therapy for OA, as several studies demonstrated catabolic and pro-inflammatory effects of IL-6 trans-signalling in cartilage and synovial tissue [72, 88, 92, 93]. Thus, even if tocilizumab does not prove effective in hand OA, specific inhibition of IL-6 trans-signalling may hold additional promise. Nonetheless, additional pre-clinical research will first be needed as the relevance of IL-6 trans-signalling in experimental OA has not yet been demonstrated.

Conclusions and future perspectives

In this review, we focus on the unique ability of IL-6 to signal via the classic- and trans-signalling pathway, and discuss the opposing effects of these signalling routes

in OA pathophysiology and treatment. Levels of IL-6 are increased in SF and serum of OA patients, and relate to disease incidence and pathology. In contrast, regulation of sIL-6R in OA, which controls activation of IL-6 trans-signalling, has been overlooked until now and warrants further research. In local joint tissues such as cartilage, synovium and bone, IL-6 classic signalling results mainly in protective effects, while trans-signalling leads to pro-inflammatory and catabolic effects. However, it is highly likely that local regulation of IL-6R levels also determines IL-6 outcome to a great extent. Current evidence of IL-6 blockade in experimental OA shows that therapeutic targeting of the IL-6 pathway could be a promising treatment strategy to reduce cartilage damage, synovial inflammation and subchondral bone pathology in OA patients. Moreover, we propose that specific blockade of IL-6 trans-signalling could be a superior treatment

strategy, which may result in inhibition of deleterious IL-6 effects in OA, while maintaining protective IL-6 signalling via the classic pathway.

Search strategy

Articles were selected using the PubMed search engine. To select articles regarding IL-6 in OA, we used following search terms present in title or abstract [tiab]: 'interleukin-6' in combination with search terms covering the topics in this review including 'osteoarthritis', 'cartilage', 'synovium', 'bone', 'muscle', 'infrapatellar fat pad' and 'therapy'. Relevant synonyms were included using MeSH terms. Title and abstract of articles were screened for relevant topics as listed in this review. Non-English articles, and articles in which IL-6 was only used as a marker of inflammation were excluded. In addition, reference lists of cited articles and articles in our personal databases were screened for eligibility. Search includes articles published up to January 2020.

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