Interleukin 4 promotes anti-inflammatory macrophages that clear cartilage debris and inhibits osteoclast development to protect against osteoarthritis

Ericka P. von Kaeppler, Qian Wang, Harini Raghu, Michelle S. Bloom, Heidi Wong, William H. Robinson

Institute for Stem Cell Biology and Regenerative Medicine, Stanford University, Stanford, CA, United States of America
VA Palo Alto Health Care System, Palo Alto, CA, United States of America
Division of Immunology and Rheumatology, Stanford University, Stanford, CA, United States of America

ARTICLE INFO

Keywords:
Osteoarthritis
Inflammation
Interleukin-4
Myeloid
Macrophage
Osteoclast

ABSTRACT

Objective: Osteoarthritis (OA), the leading cause of joint failure, is characterized by breakdown of articular cartilage and remodeling of subchondral bone in synovial joints. Despite the high prevalence and debilitating effects of OA, no disease-modifying drugs exist. Increasing evidence, including genetic variants of the interleukin 4 (IL-4) and IL-4 receptor genes, implicates a role for IL-4 in OA, however, the mechanism underlying IL-4 function in OA remains unknown. Here, we investigated the role of IL-4 in OA pathogenesis.

Methods: Il4-, myeloid-specific-Il4ra-, and Stat6-deficient and control mice were subjected to destabilization of the medial meniscus to induce OA. Macrophages, osteoclasts, and synovial explants were stimulated with IL-4 in vitro, and their function and expression profiles characterized.

Results: Mice lacking IL-4, IL-4Ra in myeloid cells, or STAT6 developed exacerbated cartilage damage and osteophyte formation relative to WT controls. In vitro analyses revealed that IL-4 downregulates osteoarthritis-associated genes, enhances macrophage phagocytosis of cartilage debris, and inhibits osteoclast differentiation and activation via the type I receptor.

Conclusion: Our findings demonstrate that IL-4 protects against osteoarthritis in a myeloid and STAT6-dependent manner. Further, IL-4 can promote an immunomodulatory microenvironment in which joint-resident macrophages polarize towards an M2 phenotype and efficiently clear pro-inflammatory debris, and osteoclasts maintain a homeostatic level of activity in subchondral bone. These findings support a role for IL-4 modulation of myeloid cell types in maintenance of joint health and identify a pathway that could provide therapeutic benefit for osteoarthritis.

1. Introduction

Osteoarthritis (OA), the most common type of arthritis, is a debilitating joint disease for which no disease-modifying therapy is available [1]. The rapidly increasing incidence of osteoarthritis due in part to the aging population and obesity epidemic [2] highlights the need to further understand its underlying pathobiology in order to develop disease-modifying therapeutics.

Osteoarthritis was previously thought to be a mechanical ‘wear-and-tear’ disease. However, accumulating evidence implicates chronic, low-grade inflammation as a key driver of osteoarthritis development, including identification of inflammatory mediators in a number of osteoarthritis tissues [3]. Further, the presence of synovitis, characterized by immune cell infiltration into the synovium, is considered a hallmark of osteoarthritis and correlates with clinical symptoms, disease severity [3] and progression of structural disease [4].

During the past decade, interleukin 4 (IL-4), a type 2 inflammatory cytokine best known for its roles in promoting atopic disease and tissue healing, has been linked to osteoarthritis. Genomic analyses identified associations between single nucleotide polymorphisms (SNPs) in the IL-4 receptor (IL4R) gene and hand osteoarthritis [5], knee osteoarthritis [6], and hip osteoarthritis [7]. SNPs in the IL-4 (IL4) gene, have also been associated with knee osteoarthritis [8]. Further, levels of soluble IL-4 receptor, an IL-4 modulator, are elevated in serum from patients with osteoarthritis [9].

Despite these genetic associations, the underlying mechanism for IL-
4 in osteoarthritis pathogenesis has yet to be explained. Multiple IL-4-responsive cell types, including macrophages and osteoclasts, are present in the joint and are known to be dysregulated in osteoarthritis. Further, macrophages and macrophage-related cytokines and chemokines have been implicated in osteoarthritis pathogenesis [10–12], and activated macrophages are present in multiple tissues of human osteoarthritic joints [13]. Osteoclasts, another joint-resident myeloid cell type, have also been implicated in osteoarthritis both in animal models [14–19] and in humans [20–23]. A key role for osteoclast biology in osteoarthritis development is further supported by the emergence of osteoclast-targeting therapies [24,25].

Most cell types are capable of responding to IL-4, and it is well-established that macrophages polarize to an “anti-inflammatory” M2 phenotype in response to IL-4 [26]. These immune-modulatory M2 macrophages are associated with wound healing, debris clearance, and other anti-inflammatory responses [27]. In addition to its role in innate immunity, IL-4 also potently inhibits osteoclast differentiation and activation [28–30].

Osteoarthritis pathogenesis lays at the intersection of immune system and musculoskeletal biology, particularly in cases of osteoarthritis arising following a history traumatic joint injury which involve cycles of chronic tissue injury and repair. Thus, IL-4-dependent macrophage polarization and osteoclast inhibition could be critical determinants in osteoarthritis development. In this study, we investigate the role of IL-4 in osteoarthritis pathogenesis, and the effects of IL-4 signaling on osteoclasts and macrophages. Our findings suggest that IL-4 modulation of myeloid cell types promotes the maintenance of joint health and reveals a pathway that could provide therapeutic benefit for osteoarthritis.

2. Materials and methods

2.1. Animals

C57BL/6 J, BALB/c, IL-4−/− (BALB/c-Il4−/−/J), STAT6−/− (C-129S2-Stat6tm1Gru/J), and IL-4Ra−/− (BALB/c-Il4raα1/J) mice were purchased from The Jackson Laboratory. IL-13Ra1−/− (C57BL/6-Ill13raα1tm1m2/J) mice were cryorecovered from Taconic. Myeloid-specific IL-4Ra−/− (LysMWT/Cre(IL-4Raα1/J)) mice were provided by Aida Habtezion (Stanford University) [31].

2.2. Mouse model of osteoarthritis

All experimental and control animals were age and sex matched using 20-week-old male mice for all experiments. Destabilization of the medial meniscus (DMM) was performed as previously described [32–34]. All animal studies were performed under protocols approved by the Stanford Committee of Animal Research and in accordance with National Institutes of Health guidelines.

2.3. Study approval

Human samples studied were under protocols approved by the Stanford Institutional Review Board (IRB) and with the subjects’ written informed consent.

2.4. Human synovial explant culture

Human synovial tissue explants were obtained from the knees of patients undergoing total knee arthroplasty (TKA) for end-stage osteoarthritis, stimulated with recombinant human IL-4 (Peprotech), and subsequently harvested for gene expression analysis.

2.5. Human macrophage culture

Human macrophages were cultured from peripheral blood monocytes as previously described [35] and stimulated with recombinant human IL-4 (R&D). Supernatants were collected for cytokine quantification, and cells were harvested for gene expression analysis. Macrophages were also cultured with human osteoarthritic cartilage debris that was harvested from patients undergoing TKA and that was labeled with amine-reactive, pH-sensitive pHrodo Red dye (ThermoFisher). Macrophages were then stained for CD11b and analyzed by flow cytometry for CD11b and pHrodo Red positivity to assess phagocytic capacity. All experiments utilized three independent biologic samples, each with two technical replicates per sample, and results were repeated in three independent experiments. Data shown are means and standard deviations of biologic replicates from a representative independent experiment. The sample size used was modeled after recent publications that employ similar methodology for in vitro macrophage functional assays [35].

2.6. Mouse macrophage culture

Mouse bone marrow-derived macrophages were isolated and cultured from long bones of 8-week-old male mice according to standard protocols. Macrophages were stimulated with IL-4 and cells collected for gene expression analysis.

All experimental conditions were performed in triplicate and were repeated in at least three independent experiments each using cells harvested from new mice. Data shown are means and standard deviations for the three technical replicates from a representative experiment. All experiments were performed using age-, sex-, and background-matched controls.

2.7. Mouse osteoclast culture

Mouse bone marrow-derived osteoclasts were isolated and cultured from long bones of 8-week-old mice as previously described [36]. Osteoclasts were stimulated with IL-4, resulting cells were stained for tartrate-resistant acid phosphatase (TRAP), and supernatants were assayed for TRAP activity as previously described [37] (Sigma). Cells were collected for gene expression analysis.

All experimental conditions were performed in triplicate and were repeated in at least three independent experiments each using cells harvested from new mice. Data shown are means and standard deviations for the three technical replicates from a representative experiment. All experiments were performed using age-, sex-, and background-matched controls.

2.8. Gene expression analysis

RNA was extracted from cell and tissue lysates using RNeasy system (Qiagen) and cDNA generated using High-Capacity cDNA Reverse Transcription Kit (AppliedBiosystems). Gene expression analysis was performed by quantitative polymerase chain reaction (qPCR) using TaqMan Gene Expression Assays (AppliedBiosystems) and TaqMan probes (Supplementary Table 1). Expression levels were quantified as fold change relative to unstimulated controls, using the 2−ΔΔCt method [38].

2.9. Statistics

For two group comparisons, data were analyzed using two-tailed, unpaired Student’s t-test in Prism (Graphpad, version 8.1). For multiple group comparisons, data were analyzed using one-way ANOVA and post hoc comparison with Tukey correction in Prism. p < 0.05 was considered statistically significant.

Detailed methods are described in online supplementary materials.
3. Results

3.1. IL-4, acting on myeloid-derived cell types and through its STAT6 signaling intermediate, protects against the development of osteoarthritis in mice

To investigate the role of IL-4 in osteoarthritis pathogenesis we utilized the destabilization of medial meniscus (DMM) model, a widely-accepted murine model of osteoarthritis development induced by surgical transection of the medial meniscus [33]. We performed DMM in mice globally deficient for the Il4 gene (Il4−/−) and Il4-sufficient (Il4+/+) wild-type control mice. Twenty weeks after surgery, Il4−/− mice showed significantly exacerbated cartilage damage and osteophyte formation compared to Il4+/+ mice (Fig. 1 A and B). We also analyzed osteoarthritis development in mice deficient for the IL-4Ra subunit of the IL-4 receptor complex in myeloid cell types (LysMCreIl4rafl/fl) and in IL-4Ra-sufficient (LysMCreIl4ra+/+) wild-type control mice following DMM. Twenty weeks after surgery, LysMCreIl4rafl/fl mice showed significantly increased cartilage damage and osteophyte formation compared to LysMCreIl4ra+/+ mice (Fig. 1 C and D). Finally, we analyzed osteoarthritis development in mice deficient for Stat6 (Stat6−/−) and Stat6-sufficient mice (Stat6+/+) wild-type control mice following DMM. Twenty weeks after surgery, Stat6−/− mice showed significantly increased cartilage damage and osteophyte formation compared to Stat6+/+ mice. Scale bars in the low-magnification (upper) images, 500 μm; scale bars in the high-magnification (lower) images, 200 μm. P values were calculated using two-tailed, unpaired Student’s t-test; *p < 0.05, **p < 0.01.
Stat6+/− mice (Fig. 1 E and F). Together these data demonstrate a critical role of IL-4 in protection against post-traumatic OA, potentially through signaling in myeloid cell types.

3.2. IL-4 stimulation of human macrophages promotes M2 polarization and enhanced phagocytosis of cartilage debris

IL-4 is known to polarize macrophages towards the M2, anti-inflammatory, phenotype. We confirmed this effect, by stimulating peripheral blood monocyte cell (PBMC)-derived macrophages from healthy human donors (n = 3) with recombinant IL-4 protein. Analysis of expression of genes and secretion of cytokines known to be associated with the M1 and M2 phenotypes revealed that 24 h after stimulation of PBMC-derived macrophages with recombinant IL-4 protein, expression of the M2-associated gene CD206 was increased over unstimulated control cells (Fig. S1A). Similarly, production of the M2-associated cytokines CCL24 and CCL18 [39] was increased in IL-4-stimulated macrophages as compared to unstimulated control cells (Fig. S1B). Together, these results recapitulate previous findings [40] that indicate that stimulation of unpolarized macrophages with IL-4 polarizes them towards the M2 phenotype.

Cartilage debris is present in osteoarthritic synovial fluids and joint tissues, as a result of cartilage breakdown [41]. Evidence from prior studies suggest that phagocytosis of cartilage debris by macrophages, or
other cell types including phagocytic chondrocytes, may be protective against osteoarthritis development [42,43]. We tested whether cartilage debris can promote polarization and phagocytic function of macrophages, by exposing PBMC-derived macrophages from healthy human donors to cartilage debris derived from end-stage osteoarthritic joints. Exposure of unpolared macrophages to osteoarthritis joint cartilage debris resulted in elevated expression of the M1-associated genes IL1b and TNFa, and decreased expression of the M2-associated genes CD206 and CD163 (Fig. 2A). These findings suggest that cartilage debris is a proinflammatory stimulus that promotes the M1 macrophage phenotype associated with production of pro-inflammatory cytokines, typically for host defense against intracellular pathogens [40].

Upon finding that cartilage debris induces the M1 phenotype in macrophages, we next assessed whether polarization to the M2 phenotype, prior to exposure to cartilage debris, was sufficient to maintain the M2 phenotype in response to a pro-inflammatory stimulus. We stimulated PBMC-derived human macrophages from healthy donors (n = 3) with recombinant IL-4 protein followed by exposure to cartilage debris derived from end-stage osteoarthritic joints. Following exposure to osteoarthritis cartilage debris, IL-4-stimulated macrophages exhibited decreased expression levels of the M1-associated gene TNFa as compared to macrophages that were not stimulated by IL-4 (unstimulated; US) prior to exposure to cartilage debris (Fig. 2B). Further, IL-4-stimulated macrophages had increased expression levels of the M2-associated gene CD206, following exposure to cartilage debris, as compared to macrophages that were unstimulated prior to exposure to cartilage debris (Fig. 2C). Similarly, macrophages that were pre-stimulated with IL-4 had increased levels of production of the M2-associated cytokines, CCL24 and CCL18, as compared to macrophages that were unstimulated prior to exposure to cartilage debris (Fig. 2D-E). These findings suggest that the M2 phenotype can be maintained in the presence of a pro-inflammatory stimulus, if exposed to IL-4 prior to exposure to the stimulus.

We further analyzed the potential function of M2 macrophages in osteoarthritis by assessing whether IL-4 stimulation of macrophages could enhance phagocytosis of cartilage debris. We treated PBMC-derived human macrophages from healthy donors (n = 3) either with or without recombinant IL-4 protein, followed by exposure to osteoarthritic cartilage debris that was covalently labeled with a pH-sensitive fluorescent dye (pHrodo Red). Flow cytometry was used to quantify all macrophages (CD11b+) as well as macrophages that took up cartilage debris through phagocytosis (CD11b+, pHrodo Red-) (Fig. 2F). Nearly all IL-4-stimulated macrophages stained positive for pHrodo Red, a significant increase as compared to the approximately half of unstimulated macrophages that stained positive for pHrodo Red following exposure to the labeled cartilage debris (Fig. 2G). Additionally, among CD11b+pHrodo' cells (termed ‘eaters’), IL-4 stimulated macrophages phagocytosed significantly more cartilage debris than unstimulated macrophages, as measured by mean fluorescence intensity (MFI) of pHrodo Red staining (Fig. 2G). This result suggests that phagocytosis of cartilage debris is stimulated to a greater degree in macrophages that have been exposed to IL-4.

3.4. IL-4 potently inhibits murine osteoclast formation in a dose-dependent manner

IL-4 is known to potently inhibit osteoclast formation [28]. To validate this effect of IL-4 on osteoclasts, we stimulated mouse bone marrow (BM)-derived osteoclasts with recombinant IL-4 over six days of differentiation (Fig. 4A). Tartrate-resistant acid phosphatase (TRAP) staining revealed robust formation of multinucleated, TRAP-positive (TRAP+) osteoclasts in the unstimulated condition, while very few multinucleated, TRAP+ osteoclasts were generated in the IL-4-stimulated condition (Fig. 4B). Quantification of TRAP in cell culture supernatants revealed that IL-4 stimulation significantly inhibited TRAP secretion from osteoclasts (Fig. 4C). qPCR analysis revealed that IL-4 stimulation resulted in decreased expression of the Il4ra, Il2rg, and Il13ra1 genes, which comprise subunits of the IL-4 receptor complexes (Fig. 4D). IL-4 stimulation also resulted in decreased expression of the osteoclast differentiation-associated genes Nfatc1, Trp53, and Acss3 (Fig. 4E) and osteoclast activation genes Ctsk, Mmp9, Calcr, and Cat2 (Fig. 4F).

We also investigated the potency of IL-4 in inhibition of osteoclastogenesis by testing multiple doses of recombinant IL-4 stimulation. Stimulation with either 1 ng/mL or 10 ng/mL recombinant IL-4 resulted in lower measurable supernatant TRAP as compared to 0.1 ng/mL IL-4. No significant differences in supernatant TRAP levels were observed between stimulation with either 1 ng/mL or 10 ng/mL (Fig. 4G). Similarly, stimulation with either 1 ng/mL or 10 ng/mL recombinant IL-4 resulted in dramatic reductions in the expression of the osteoclast differentiation gene, Acp5, and the osteoclast activation gene, Ctsk, as compared to unstimulated osteoclasts, while stimulation with 0.1 ng/mL IL-4 resulted in relatively less inhibition of these genes (Figs. 4 H and I). These findings suggest that IL-4 can inhibit osteoclast differentiation and activation in a dose-dependent manner.

We further investigated the role of these effects of IL-4 on osteoclasts observed in vitro in our in vivo DMM model of post-traumatic OA through immunohistochemical staining of the monocyte marker Cd68 [46] and osteoclast activity marker cathepsin K [47]. Il4−/− mice showed increased Cd68+ osteoclasts (Fig. 4J) and cathepsin K (Fig. 4K) staining compared to wild-type control mice following DMM. These findings demonstrate that osteoclast number and activity are increased in osteoarthritic joints of IL-4 deficient mice, reinforcing the relevance of our in vitro findings.

3.5. IL-4 inhibits osteoclast formation via the type 1 receptor

We further parsed the role of these effects of IL-4 on osteoclasts in vitro in our in vivo DMM model of post-traumatic OA through immunohistochemical staining of the monocyte marker Cd68 [46] and osteoclast activity marker cathepsin K [47]. Il4−/− mice showed increased Cd68+ osteoclasts (Fig. 4J) and cathepsin K (Fig. 4K) staining compared to wild-type control mice following DMM. These findings demonstrate that osteoclast number and activity are increased in osteoarthritic joints of IL-4 deficient mice, reinforcing the relevance of our in vitro findings.
multinucleated, TRAP$^+$ osteoclasts in cells derived from WT and Il13ra$^{-/-}$ mice (Fig. 5 A and B), but not in cells derived from Il4ra$^{-/-}$ mice (Fig. 5C). Quantification of cell culture supernatants revealed significantly decreased levels of TRAP in IL-4-stimulated cells derived from WT and Il13ra$^{-/-}$ mice (Fig. 5D and E) as compared to unstimulated cells. Levels of supernant TRAP were also decreased in cells derived from Il4ra$^{-/-}$ mice, but to a lesser extent than observed in WT and Il13ra$^{-/-}$ cells (Fig. 5F).

We further tested the role of IL-4 in osteoclastogenesis by looking at its effect on expression of genes involved in the regulation of osteoclast differentiation and activation. IL-4 stimulation led to decreased levels of the osteoclast differentiation genes Tnaf1, Tnfsf11a and Acp5 in cells derived from WT and Il13ra$^{-/-}$ mice (Fig. 5 G and H) but not in cells derived from Il4ra$^{-/-}$ mice (Fig. 5I). Similarly, genes associated with osteoclast activation, Ctsk, Mmp9, Calcr, and Car2 exhibited significantly decreased expression in cells derived from WT and Il13ra$^{-/-}$ mice following stimulation with IL-4 stimulation (Fig. 5 J and K), while we did not observe significant changes in the expression of these genes following IL-4 stimulation of cells derived from Il4ra$^{-/-}$ mice (Fig. 5L). Together these findings indicate that IL-4 likely signals through the IL-4R$^+$ receptor complex in osteoclasts and may mediate its effects in osteoarthritis.

3.6. IL-4 pathway components are dysregulated in human osteoarthritis synovium

We next sought to determine whether IL-4 signaling was dysregulated in human osteoarthritis joints. We analyzed IL-4 protein levels in synovial fluids derived from human subjects with osteoarthritis ($n = 12$) and non-osteoarthritis arthropathies (rheumatoid arthritis $n = 4$, psoriatic arthritis $n = 2$, reactive arthritis $n = 1$, and crystal-induced arthritis $n = 2$) by ELISA. The concentration of IL-4 was significantly higher in synovial fluids derived from individuals with osteoarthritis as compared to synovial fluids from individuals with non-osteoarthritis arthritides ($p < 0.01$; Fig. 6A).

We also investigated the effect of IL-4 on expression of inflammatory and degradative mediators in human synovial tissues, as macrophages reside in this tissue. We stimulated human osteoarthritis synovial tissue explants with recombinant IL-4 protein for 24 h. qPCR analysis revealed that IL-4 stimulation resulted in decreased expression of a panel of cytokine, chemokine, and enzyme genes known to be associated with osteoarthritis (Fig. 6B). These findings suggest that IL-4 expression in synovial tissues may downregulate expression of mediators that contribute to joint dysfunction in osteoarthritis.

4. Discussion

The objective of this study was to investigate the role of IL-4 in the development of osteoarthritis. The association of SNPs in the IL4 and IL4Ra genes [5–8], along with the dysregulation of IL-4 in osteoarthritis synovial fluid suggest that this pathway, typically associated with Type 2 immunity and atopic diseases, plays a role in the development of osteoarthritis. Here we show that, in a mouse model of post-traumatic osteoarthritis, IL-4 deficiency exacerbates disease with worse cartilage damage and osteophyte formation. Further, we show that STAT6 deficiency and IL-4Ra deficiency in myeloid cells also exacerbates osteoarthritis in mice. Taken together, these data suggest that IL-4 protects against osteoarthritis via signaling through its IL-4Ra subunit and its STAT6 signaling intermediate in myeloid cell types.

In vitro analyses of IL-4 effects on human synovium and joint-resident myeloid cell types revealed that IL-4 can inhibit osteoarthritis-related phenotypes. In macrophages, IL-4 stimulates improved phagocytosis of cartilage debris, a potential mechanism by which macrophages can modulate the DAMP-driven inflammatory microenvironment of a
diseased joint. Further, IL-4 stimulates M2 polarization of macrophages via the type I receptor, even in the presence of cartilage debris that would otherwise be an M1 stimulus. Though the exact polarization state of macrophages in an osteoarthritis joint remains unclear, the effects of IL-4 on macrophages may be essential for maintaining tissue homeostasis and in the damage response in synovial joints, similar to its known role in other tissue types [48].

IL-4 stimulation of osteoclasts confirmed previous findings that IL-4 potently inhibits osteoclast formation. We extended these findings by using receptor subunit-deficient osteoclasts to demonstrate that IL-4 uses the type I receptor to downregulate expression of osteoclast differentiation and activation genes. Given the known role of osteoclast overactivation in osteoarthritis pathogenesis [20–23], these data suggest that, in a healthy joint, IL-4 can prevent pathogenic differentiation and activation of osteoclasts. Further, the finding that IL-4 acts through the same receptor complex in both macrophages and osteoclasts highlights the potential role of the type I receptor in mediating joint-protective functions through a common mechanism in both cell types.

Fig. 4. IL-4 potently and dose-dependently inhibits murine osteoclast formation.
(A) Experimental design of osteoclast stimulation with recombinant IL-4 throughout six days of differentiation. (B) TRAP staining of unstimulated (US) and IL-4 stimulated (+IL-4) osteoclasts. (C) Quantification of supernatant TRAP activity from US and + IL-4 osteoclasts. TRAP activity is represented as OD540, relative to the US condition. (D–F) show expression of IL-4 receptor subunits (D), osteoclast differentiation-associated genes (E), and osteoclast activation-associated genes (F), as measured by quantitative PCR analysis of relative mRNA expression, in US or + IL-4 osteoclasts. (G–I) Dose response of osteoclast to stimulation with recombinant IL-4, as measured by supernatant TRAP activity (G), osteoclast differentiation-associated gene expression (H), and osteoclast activation-associated gene expression (I). (J–K) Representative low-magnification (upper) and high-magnification (lower) immunohistochemical staining of osteoclasts markers CD68 (J) and cathepsin K (K) in the femoro-tibial joint from wild type (WT) and IL-4 deficient (IL-4−/) mice subjected to DMM. Scale bars in the images, 200 μm. Gene expression is reported as fold change over the unstimulated condition. For 2 group comparisons, P values were calculated using two-tailed, unpaired Student’s t-test; *P < 0.05, **P < 0.01. For multiple group comparisons, P values were calculated using one-way ANOVA and post hoc comparison with Tukey correction; *P < 0.05, **P < 0.01.
The implication of osteoclast dysregulation as one mechanism by which IL-4 modules the joint environment highlights this osteoimmunologic axis as a potentially relevant target for pharmacologic intervention, either at the level of the osteoclast or the IL-4 cytokine. Many studies to date have investigated the effects of osteoclast-specific pharmacologics including bisphosphonates, calcitonin, cathepsin K inhibitors, and estrogen replacement therapy on osteoarthritis development [21,24]. While select animal models have shown small
beneficial effects with early osteoclast inhibition with bisphosphonates, multiple animal model studies and human trials have largely failed to show significant benefit of bisphosphonates in the prevention or treatment of osteoarthritis [24]. The lack of success with these osteoclast-specific agents likely highlights the multifactorial nature of osteoarthritic pathogenesis and, thus, the need to target multiple pathologic cell types, potentially using IL-4.

Though more often studied in models of atopic and other T-cell mediated disease than in osteoarthritis-specific models, pharmacologic targeting of IL-4 has been investigated with both anti-cytokine [49] and cytokine enhancement [50] strategies. Recombinant IL-4 has been found to be protective in models of inflammatory arthritis [51] and peri-implant osteolysis [52]. In models of osteoarthritis, intra-articular injection of IL-4 in rats [53] and systemic treatment with IL-4-10 fusion protein in canines [54] protect against cartilage damage, highlighting the therapeutic potential of this cytokine. Recent advances in IL-4 therapeutics have developed chemically modified IL-4 ‘superkines’, with enhanced cytokine affinity and receptor specificity, that may underlie protection against osteoarthritis. IL-4 has been shown to be protective, such as in wound repair [48]. Further investigation is needed to assess the effects of supraphysiologic levels of IL-4 in the joint and to investigate other approaches to promote this protective mechanism in osteoarthritis.

The observations presented here suggest a potential, novel therapeu- tic strategy of using recombinant IL-4 or activators of the IL-4R signaling pathway to treat osteoarthritis. Currently, therapeutic recombinant IL-4 is being investigated for the prevention of peri-prosthetic osteolysis [52]. Given the type 1 receptor specificity reported here, the use of type I receptor-targeted engineered IL-4 superkines, as described by Junttila et al. [50], might also prove useful in developing the first disease-modifying treatment for osteoarthritis. Further studies will be needed to assess the effects of supraphysiologic levels of IL-4 in the joint and to investigate other approaches to promote this protective mechanism in osteoarthritis.

**Funding**

The research was supported by VA Merit Awards I01RX002689 and BX004713-01A1 to W.H.R.

**Declaration of Competing Interest**

The authors have declared that no conflict of interest exists.

**Acknowledgments**

The authors would like to thank Eileen Elliott for her work processing and banking human osteoarthritis tissue samples and Amira Barkal for her assistance in the setup of the macrophage phagocytosis assay. We also thank Drs. Mary Nakamura, Joy Wu, and Srilatha Swami for their input and technical assistance in the setup of osteoclast culture system.

**Appendix A. Supplementary data**

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clim.2021.108784.

**References**


