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Brief report
Elevated serum levels of soluble interleukin-4 receptor in osteoarthritis

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Summary

Objective: To test the importance of the interleukin-4 (IL-4)/IL-4 receptor (IL-4R) system in osteoarthritis (OA) we evaluated soluble IL-4R (sIL-4R) levels in sera of patients with different forms of OA and healthy individuals.

Methods: We recruited: 141 patients with hand OA, 70 with nodal and 71 with erosive hand OA; 64 patients undergoing total joint replacement, 34 with hip and 30 with knee OA; and 38 ethnically and geographically age-matched healthy individuals [normal controls (NC)].

Results: Serum sIL-4R concentration was found to be significantly higher in all OA patients than that in NC. When patients were divided into four subgroups (nodal, erosive, hip and knee OA) significant differences were present when comparing NC with each subgroup. This was true also when small-joint OA groups were compared with large-joint OA groups, the latter being associated with higher IL-4R levels.

Conclusions: We found increased levels of sIL-4R in OA patients compared with healthy individuals. We speculate that this reduces availability of IL-4, and its effects on chondrocytes.

Key words: Osteoarthritis, Interleukin-4, Soluble receptor.

Introduction

Osteoarthritis (OA) is a degenerative joint disease characterised by a loss of balance between the anabolic and catabolic functions of chondrocytes. Enhanced breakdown of cartilage matrix and reduced synthesis of matrix components by articular chondrocytes eventually lead to the destruction of the affected tissue[1]. Cytokines and growth factors seem to be involved in regulating cartilage degradation as well as synthesis[2]. Interleukin-1 beta (IL-1β) and tumour necrosis factor alpha (TNF-α) have been shown to inhibit chondrocyte proteoglycan synthesis, while interleukin-4 (IL-4), interleukin-10 (IL-10) and interleukin-13 (IL-13) have been shown to prevent and reverse cartilage degradation *in vitro*[3].

IL-4 exhibits potent anti-inflammatory activities by inhibiting synthesis of IL-1β and TNF-α and by down regulating many effects of these cytokines[4]. IL-4 induces its cellular responses by binding to a multimeric receptor. The primary subunit of the IL-4 receptor (IL-4R), the α subunit, can combine with the common gamma chain of the interleukin-2 receptor or the α′ subunit of the IL-13 receptor. Thus, different IL-4Rs can be found in different cells. Human IL-4Rα exists either as a membrane-bound receptor (IL-4R), or as soluble one [soluble IL-4R (sIL-4R)][5]. The latter is produced by proteolytic shedding of the membrane-bound form[6], or by alternative splicing of the gene[5]. Depending on its molar ratio to IL-4, sIL-4R can augment or neutralise IL-4 *in vitro*[7]. To assess whether the IL-4/IL-4R system was involved in OA, we measured soluble IL-4R levels in sera of patients with different forms of OA.

Methods

Measurements were made in four groups of patients with OA and a control group. The first two groups were recruited among consecutive men and women who attended six rheumatological units in Italy, with a special interest in OA. Patients with hand OA were diagnosed following clinical and radiological evaluations[8]. Subjects had evidence of hard tissue enlargement and/or deformity in three or more index hand joints. Hand radiographs were used to subdivide hand OA patients into nodal and erosive groups. Erosive OA was defined by central erosions ("gull-wing" erosions) and/or ankylosis in the interphalangeal joints in at least three digits, associated with joint-space narrowing, subchondral sclerosis and/or osteophytes. We recruited 141 patients (eight male, 133 female; mean age 62.4 years (yrs), SD ± 9.2, range 44–85 yrs), 70 with nodal (three male, 67 female; mean age 59 yrs, SD ± 9.6, range 44–85 yrs) and 71 with erosive (five male, 66 female; mean age 64.4 yrs, SD ± 8.3, range 44–82 yrs) hand OA. Less than 30% of the patients were presented with manifestations of OA at the spine, hip or knee. Patients with a history of other rheumatic or skeletal diseases were excluded from analysis. In particular, for patients with erosive OA,

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subjects with psoriasis or a family history of psoriasis were excluded.

The third and fourth groups were recruited from consecutive men and women admitted to the Istituti Ortopedici Rizzoli in Bologna (Italy) for total joint replacement. These patients were diagnosed, following clinical and radiological evaluations, as having either idiopathic hip or knee OA without known metabolic, traumatic, congenital or endocrine causes[9,10], with a Kellgren–Lawrence radiological score of 3–4. We recruited 64 patients (21 male, 43 female; mean age 69.5 yrs, SD ± 8.4, range 39–86 yrs), 34 with hip OA (15 male, 19 female; mean age 67.6 yrs, SD ± 9.8, range 39–86 yrs) and 30 with knee OA (six male, 24 female; mean age 71.6 yrs, SD ± 5.8, range 57–81 yrs). Patients with a history of other rheumatic or skeletal diseases were excluded from analysis. Patients were classified radiographically as described by Altman *et al.*[11]. For each patient clinical, radiological and laboratory data were collected, and coded for confidentiality. We also analysed 38 ethnically and geographically age-matched healthy individuals (eight male, 30 female; mean age 66.7 yrs, SD ± 9.5, range 37–79 yrs), fulfilling the admission criteria of the SENIEUR protocol[12]. Clinical evidence of OA at the hand, knee or hip was excluded. Informed consent was obtained from each patient. The study was approved by the ethics committees of the hospitals involved. For each individual enrolled in the study, venous blood was drawn for serum and plasma separation. Soon after collection, vacutainers were centrifuged at 2000 rpm for 10 min in order to obtain serum and plasma, which were stored at −80 °C for further analysis. sIL-4R serum concentrations were measured by immunoassay using a commercial enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Minneapolis, MN), according to the manufacturer's instructions. The detection limit was 5 pg/mL.

STATISTICAL ANALYSES

Continuous variables were subjected to a Kruskal–Wallis test and a Mann–Whitney *U* test for unpaired data. Regression analyses and Spearman *R* correlation tests were also performed.

Results and discussion

Serum sIL-4R concentrations in all OA patients were increased in comparison with normal controls (NC) (NC = 25.6 pg/mL (median), total OA = 35.7 pg/mL (median), $P < 0.001$). When patients were divided into four groups (nodal, erosive, hip and knee OA) significant differences ($P < 0.001$) from controls were present for each comparison, but there were no differences between nodal and erosive OA, and hip vs knee OA (Fig. 1). This was true also when small-joint OA groups were compared with large-joint OA groups, the latter being associated with higher sIL-4R levels. Regression analysis and correlations showed no significant association between either sex or age and sIL-4R serum levels in all groups.

In this study, we measured the concentration of sIL-4R in the sera of patients with primary OA at the hand, hip or knee. We showed that sIL-4R levels were significantly increased in sera from patients compared with controls. Levels of sIL-4R were increased in comparison with controls in the whole group of OA patients as well as in the subgroups with hand, hip and knee OA. IL-4 is expressed in normal articular cartilage and has chondroprotective

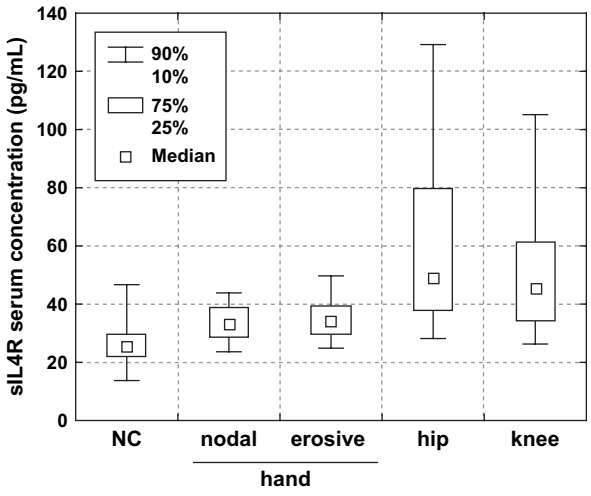

Fig. 1. Serum levels of sIL-4R in patients with nodal OA, erosive OA, hip OA, knee OA and in healthy individuals (NC). Squares show median, boxes 25th and 75th percentiles, and whiskers 10th and 90th percentiles. Significant difference ($P < 0.001$) was present for each comparison, except for nodal vs erosive OA, and hip vs knee OA.

effects *in vitro*, inhibiting IL-1-induced production of metalloproteinases by chondrocytes[13]. IL-4 is also involved in mechanotransduction in normal human articular chondrocytes[14], via an autocrine/paracrine loop. IL-4 is produced by the cell in response to mechanical stimulation, binds to receptors on chondrocytes, and drives a signalling cascade leading to activation of ion channels and aggrecan synthesis. Optimal mechanical stimulation maintains articular cartilage structure and function, whereas abnormal mechanical forces lead to loss of cartilage and the onset of OA. Chondrocytes from OA cartilage do not show the same response to mechanical stimulation as normal cells[14]. Whatever the mechanism involved, in OA, the anabolic and protective effect of IL-4 seems to fail. This could be caused by an alteration of the IL-4/IL-4R system at any level: reduction of IL-4 action/availability, receptor down-regulation, or signalling impairment. It has been shown that low concentrations of sIL-4R, compared with those of IL-4, enhance IL-4 activity, while higher concentrations have the opposite effect[7]. This may be involved in the control of IL-4 activity in normal conditions. sIL-4R produced by alternative splicing[5] is probably regulated by IL-4 via STAT, as it is in mice[15], while production following proteolysis is under the control of metalloproteases[6]. In OA, over-production of such enzymes has been demonstrated[1]. Thus increased production of sIL-4R in OA might be the consequence of enhanced proteolysis of the membrane form. In a recent paper by Forster *et al.*[16], two single nucleotide polymorphisms (SNPs) in the IL-4R gene have been associated with susceptibility to hip OA in a group of patients undergoing total joint replacement. Both SNPs are located in the intracellular portion of IL-4R and can affect the binding and phosphorylation of the intracellular substrates STAT6 and IRS. This indirect evidence of IL-4R involvement in OA probably exerts its effects through changes in the signalling cascade. The increased production of sIL-4R observed in patients could result in reduced availability of IL-4[7] and a reduction in the effects of IL-4 on chondrocytes. To substantiate such a hypothesis it would be necessary to measure IL-4 and IL-4R levels simultaneously in the same patients.

Furthermore, systemic sIL-4R levels may be influenced by local levels in cartilage and synovial fluid. We have demonstrated that involvement of large joints such as the hip and knee was associated with significantly higher concentrations of sIL-4R, when compared with levels in patients with hand OA. One can speculate that this could be due to differences in joint size, or disease state. In order to ascertain whether serum measurements of sIL-4R will be a useful tool for disease monitoring, longitudinal studies of patients with early as well as late OA should be performed to assess the value of sIL-4R as disease marker.

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References

1. Martel-Pelletier J. Pathophysiology of osteoarthritis. Osteoarthritis Cartilage 1999;7(4):371–3.
2. Westacott CI, Sharif M. Cytokines in osteoarthritis: mediators or markers of joint destruction? Semin Arthritis Rheum 1996;25:254–72.
3. Fernandes JC, Martel-Pelletier J, Pelletier JP. The role of cytokines in osteoarthritis pathophysiology. Biorheology 2002;39:237–46.
4. Woods JM, Katschke KJ, Volin MV, Ruth JH, Woodruff DC, Amin MA, *et al*. IL-4 adenoviral gene therapy reduces inflammation, proinflammatory cytokines, vascularization, and bony destruction in rat adjuvant induced arthritis. J Immunol 2001;166:1214–22.
5. Kruse S, Forster J, Kuehr J, Deichmann KA. Characterization of the membrane-bound and a soluble form of human IL-4 receptor alpha produced by alternative splicing. Int Immunol 1999;11(12):1965–70.
6. Idzerda RL, March CJ, Mosley B, Lyman SD, Vanden Bos T, Gimpel SD, *et al*. Human interleukin 4 receptor confers biological responsiveness and defines a novel receptor superfamily. J Exp Med 1990 Mar 1;171(3): 861–73.
7. Jung T, Wagner K, Neumann C, Heusser CH. Enhancement of human IL-4 activity by soluble IL-4 receptors *in vitro*. Eur J Immunol 1999;29:864–71.
8. Altman R, Alarcon G, Appelrouth D, Bloch D, Borenstein D, Brandt K, *et al*. The American College of Rheumatology criteria for the classification and reporting of osteoarthritis of the hand. Arthritis Rheum 1990;33:1601–10.
9. Altman R, Asch E, Bloch D, Bole G, Borenstein D, Brandt K, *et al*. Development of criteria for the classification and reporting of osteoarthritis. Classification of osteoarthritis of the knee. Diagnostic and Therapeutic Criteria Committee of the American Rheumatism Association. Arthritis Rheum 1986;29:1039–49.
10. Altman R, Alarcon G, Appelrouth D, Bloch D, Borenstein D, Brandt K, *et al*. The American College of Rheumatology criteria for the classification and reporting of osteoarthritis of the hip. Arthritis Rheum 1991;34:505–14.
11. Altman RD, Hochberg M, Murphy WA Jr, Wolfe F, Lequesne M. Atlas of individual radiographic features in osteoarthritis. Osteoarthritis Cartilage 1995;3(Suppl A):3–70.
12. Ligthart GJ, Corberand JX, Fournier C, Galanaud P, Hijmans W, Kennes B, *et al*. Admission criteria for immunogerontological studies in man: the SENIEUR protocol. Mech Ageing Dev 1984 Nov;28(1):47–55.
13. Nemoto O, Yamada H, Kikuchi T, Shinmei M, Obata K, Sato H, *et al*. Suppression of matrix metalloproteinase-3 synthesis by interleukin-4 in human articular chondrocytes. J Rheumatol 1997;24:1774–9.
14. Millward-Sadler SJ, Wright MO, Davies LW, Nuki G, Salter DM. Mechanotransduction via integrins and interleukin-4 results in altered aggrecan and matrix metalloproteinase 3 gene expression in normal, but not osteoarthritic, human articular chondrocytes. Arthritis Rheum 2000;43:2091–9.
15. Kotanides H, Reich NC. Interleukin-4-induced STAT6 recognizes and activates a target site in the promoter of the interleukin-4 receptor gene. J Biol Chem 1996; 271(41):25555–61.
16. Forster T, Chapman K, Loughlin J. Common variants within the interleukin 4 receptor alpha gene (IL4R) are associated with susceptibility to osteoarthritis. J Hum Genet 2004;114(4):391–5.