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Interleukin-1 is not involved in synovial inflammation and cartilage destruction in collagenase-induced osteoarthritis

S.C.M. van Dalen †, A.B. Blom †, A.W. Slöetjes †, M.M.A. Helsen †, J. Roth ‡, T. Vogl ‡, F.A.J. van de Loo †, M.I. Koenders †, P.M. van der Kraan †, W.B. van den Berg †, M.H.J. van den Bosch †, P.L.E.M. van Lent † *

† *Experimental Rheumatology, Department of Rheumatology, Radboud University Medical Center, Nijmegen, The Netherlands*
‡ *Institute of Immunology, University of Münster, Münster, Germany*

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SUMMARY

Objective: Interleukin-1 (IL-1) is an alleged important cytokine in osteoarthritis (OA), although the exact contribution of IL-1 to joint destruction remains unclear. Here we investigated the involvement of IL-1α and IL-1β in joint pathology during collagenase-induced OA (CiOA).
Methods: CiOA was induced in wild type (WT) and IL-1αβ$^{-/-}$ mice. Additionally, IL-1 signaling was inhibited in WT mice with CiOA using osmotic pumps containing IL-1RA. Joint pathology was assessed using histology. Activity of cartilage-degrading enzymes was determined using antibodies against aggrecan neo-epitopes VDIPEN and NITEGE. Synovial gene expression was analyzed using quantitative real-time polymerase chain reaction (qRT-PCR). Serum protein levels were measured with Luminex or enzyme-linked immunosorbent assay (ELISA).
Results: Synovial IL-1β expression was strongly elevated 7 days after induction of CiOA in WT mice but decreased afterwards, whereas S100A8/A9, previously described to aggravate OA, remained elevated for 21 days. Remarkably, synovial inflammation was comparable between WT and IL-1αβ$^{-/-}$ mice on day 7 of CiOA. In line, synovial mRNA expression of genes involved in IL-1 signaling and inflammatory mediators was comparable between WT and IL-1αβ$^{-/-}$ mice, and serum levels for Keratinocyte Chemoattractant (KC)/IL-6/S100A8/S100A9/IL-10 were equal. Synovial matrix metalloproteinase (MMP)/aggrecanase expression and activity in cartilage was not different in WT and IL-1αβ$^{-/-}$ mice on day 7 of CiOA. Cartilage destruction on day 42 was not different between WT and IL-1αβ$^{-/-}$ mice, which was supported by our finding that IL-1RA treatment in WT mice with CiOA did not alter joint destruction.
Conclusions: IL-1α and IL-1β are not involved in synovial inflammation and cartilage destruction during CiOA, implicating that other mediators are responsible for the joint damage.

Introduction

Osteoarthritis (OA) is a degenerative disease of the entire joint, characterized by severe cartilage destruction, fibrosis and ectopic bone formation. Recently, the development of joint erosions was associated with persistent synovitis in hand OA[1]. Up to 50% of OA patients show low grade joint inflammation[2,3], which is reflected by a thickened synovial lining, activation of resident cells and elevated release of macrophage-derived inflammatory mediators, such as interleukin-1 (IL-1) and the alarmins S100A8 and S100A9[4,5].

Our lab previously described the involvement of S100A8/A9 in the etiopathology of OA[6,7], but the exact contribution of IL-1 to OA pathology is still unclear. The IL-1 family members IL-1α and IL-1β have been shown to be present in synovial fluid and articular cartilage in pigs with OA, and correlate with joint destruction[8]. IL-1β levels are not only elevated in animal models of experimental OA, also in OA patients IL-1 proteins are found to be increased[9,10].

* Address correspondence and reprint requests to: P.L.E.M. van Lent, Radboud University Medical Center, Geert Grooteplein 28, 6525 GA Nijmegen, The Netherlands. Fax: 31-243540403.
E-mail addresses: Stephanie.vanDalen@radboudumc.nl (S.C.M. van Dalen), Arjen.Blom@radboudumc.nl (A.B. Blom), Annet.Sloetjes@radboudumc.nl (A.W. Slöetjes), Monique.Helsen@radboudumc.nl (M.M.A. Helsen), rothj@uni-muenster.de (J. Roth), vogl@uni-muenster.de (T. Vogl), Fons.vandeLoo@radboudumc.nl (F.A.J. van de Loo), Marije.Koenders@radboudumc.nl (M.I. Koenders), Peter.vanderKraan@radboudumc.nl (P.M. van der Kraan), Wim.vandenBerg@radboudumc.nl (W.B. van den Berg), Martijn.vandenBosch@radboudumc.nl (M.H.J. van den Bosch), Peter.vanLent@radboudumc.nl (P.L.E.M. van Lent).

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IL-1α and IL-1β are encoded by distinct genes but have similar biological, agonistic properties which are extensively reviewed by Garlanda *et al.*[11] Both proteins are produced independently by immune cells, such as activated tissue macrophages in response to Toll-like receptor (TLR) signaling[12]. IL-1α and IL-1β bind to the same receptor, IL-1R1, which results in pro-inflammatory signaling via recruitment of downstream cytoplasmic adapter proteins like myeloid differentiation primary response gene 88 (MYD88) by the Toll/interleukin-1 receptor (TIR) domain. Negative regulation of IL-1 signaling runs via the naturally occurring soluble receptor antagonist IL-1RA, which competes for binding sites on IL-1R1[13], and via the soluble decoy receptor IL-1R2, which binds both IL-1α and IL-1β, but lacks the TIR domain and therefore does not signal.

The fully active IL-1α precursor is very potent in degrading proteoglycans in cartilage[14,15] and inhibiting proteoglycan synthesis by chondrocytes[16]. A deletion polymorphism at the miRNA-122 binding site in the 3′UTR (untranslated region) of IL-1α is associated with an increased risk for OA[17]. Activation of IL-1β takes place in a protein complex referred to as the inflammasome, through cleavage of pro-IL-1β by caspase-1[18], followed by release of the active cytokine into the extracellular space where it exerts similar processes as IL-1α. IL-1β causes the degeneration of articular cartilage by inducing the expression of matrix metalloproteinase (MMP)-1 and MMP-13[19,20], and aggrecanase-1 and -2 (a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS)-4 and -5)[21,22] in chondrocytes and synovial fibroblasts. Moreover, it has been shown that IL-1β reduces the synthesis of the matrix components type II collagen and proteoglycans by chondrocytes[23,24].

Since both IL-1α and IL-1β play an important role in cartilage degradation, blocking IL-1 appears to be a promising therapeutic approach to diminish joint destruction in OA patients. *In vitro*, inhibition of the IL-1-induced phosphorylation of the mitogen-activated protein (MAP) kinases extracellular-signal regulated kinase (ERK), protein 38 (p38) and c-Jun N-terminal kinase (JNK), results in down-regulation of MMP gene expression in chondrocytes[25]. However, inhibition of IL-1 signaling with IL-1RA in OA patients gave disappointing results[26], although several clinical trials are still ongoing.

In the present study, we investigated the role of IL-1α and IL-1β in the etiopathology of the collagenase-induced OA (CiOA) mouse model, which has a clear inflammatory component that is involved in the development of the pathology. We hypothesized that IL-1 aggravates joint pathology in CiOA by induction of synovitis and cartilage destruction, and that blocking IL-1 signaling will prevent this. Therefore, we used a mouse strain deficient in IL-1α and IL-1β to elucidate the contribution of these pro-inflammatory proteins to synovial inflammation and the expression of other inflammatory mediators. Moreover, we studied the formation of the aggrecan neo-epitopes VDIPEN and NITEGE in cartilage in the absence of IL-1α and IL-1β and whether this resulted in reduced cartilage damage. Finally, we inhibited IL-1 signaling during CiOA using IL-1RA as an alternative approach to support our findings.

Methods

Animals

Female C57BL/6 mice of 12 weeks old were obtained from Janvier (Le Genest Saint Isle, France). IL-1αβ$^{-/-}$ mice in a C57BL/6 background[27] were a kind gift of Dr Y. Iwakura (University of Tokyo, Center of Experimental Medicine, Tokyo, Japan) and were bred in house. Females were used for experiments at the age of 12 weeks old. Group sizes were based on the expected variation per experiment. The animals were housed in filter-top cages with woodchip bedding. Mice received a standard diet and tap water *ad libitum*. All animal studies were according to the Dutch law and approved by the local Animal Experimentation Committee.

Induction of CiOA

CiOA was induced as described before in Ref. 28 by two intra-articular injections of 1 U collagenase type VII from *Clostridium histolyticum* (Sigma–Aldrich) into knee joints of wild type (WT) and IL-1αβ$^{-/-}$ mice on day 0 and day 2, causing damage to collateral and cruciate ligaments leading to local instability of the knee joint. This resulted in an OA-like phenotype with chronic synovial activation and cartilage destruction. Day 42 was taken as end point of the disease. Contralateral saline-injected knee joints were used as controls.

IL-1RA treatment

Mice with CiOA intraperitoneally received an osmotic pump (Alzet 1007D, Alza corp., Palo Alto CA, USA) with IL-1RA recombinant protein in saline. They were set to release 37.5 μg IL-1RA per hour during the first 2 weeks starting immediately after induction of CiOA as described before in Ref. 29. Control mice received an empty pump. Mice were sacrificed at day 28 after induction of CiOA.

Preparation of RNA and quantitative real-time polymerase chain reaction (qRT-PCR)

On day 7 after induction of CiOA, synovial specimens were isolated as described before in Ref. 30. Briefly, joint capsule biopsies were isolated on the medial and lateral side of the patella. Synovial biopsies were snapfrozen in liquid nitrogen and stored for RNA isolation. Synovial biopsies were homogenized in TRI-reagent (Sigma–Aldrich) using the MagNa Lyser (Roche), followed by RNA isolation according to the manufacturer's protocol. The RNA concentration was determined using a Nanodrop spectrophotometer and subsequently reverse transcribed into cDNA as previously described in Ref. 6. mRNA levels of cytokines and chemokines were detected using a StepOnePlus qRT-PCR system (Applied Biosystems) using SYBR green master mix (Applied Biosystems, Foster City, CA) and specific primers (Biolegio; Table I). Relative quantification of the qRT-PCR signals was performed by correcting the C_t value of the genes of interest for glyceraldehyde 3-phosphate dehydrogenase (GAPDH) content ($-\Delta C_t$). Contralateral knee joints without induction of experimental OA were used as negative control for the time course of the CiOA model.

Measurement of local and systemic protein levels in mice with CiOA

Washouts from synovial specimens were made by incubation of freshly isolated synovial specimens of knee joints in culture medium containing 0.1% bovine serum albumin (BSA) for 1 h at room temperature (RT). Local release of S100A8/A9 in these washouts and systemic S100A8/A9 levels in serum were quantified by a sandwich enzyme-linked immunosorbent assay (ELISA) specific for murine S100A8/A9 as described previously in Ref. 31. Protein levels were measured in serum using Luminex multianalyte technology, using the Bio-Rad Bio-Plex™ 200 System with magnetic beads specific for Keratinocyte Chemoattractant (KC), interleukin-6 (IL-6), and interleukin-10 (IL-10) according to the manufacturer's protocol (Bio-Rad).

Histological analysis of OA progression

Total knee joints of mice were fixed in 4% formalin, decalcified in 4% formic acid buffered in PBS and embedded in paraffin. Coronal sections (7 μm) were cut and stained with haematoxylin/eosin (H&E) to score synovial activation, which was identified as