Chemokines differentially induce matrix metalloproteinase-3 and prostaglandin E_2 in human articular chondrocytes

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Abstract

Objective

To explore prostaglandin (PG) E_2 production by human articular chondrocytes induced by different chemokines.

Methods

Human chondrocytes were enzymatically isolated from the articular cartilage of patients with rheumatoid arthritis (RA), osteoarthritis (OA) or traumatic fracture (N) who underwent total joint replacement. They were cultured in vitro as monolayers and then exposed to MCP-1, RANTES or SDF-1 for 24 h. Levels of PGE_2 and MMP-3 in the culture supernatant were then immunoassayed.

Results

PGE_2 production was enhanced up to 2.7-fold in a subset of samples. Responses to different chemokines were heterogeneous even within the same disease groups. As previously reported, chemokines induced MMP-3 secretion by chondrocytes, but there was no significant correlation between levels of PGE_2 and MMP-3.

Conclusion

We here document the presence of "responders" among OA, RA and normal chondrocytes that produce enhanced levels of PGE_2 upon stimulation by chemokines. The relationship between chemokines and prostaglandins could differentially influence the pathogenic network responsible for cartilage degradation in arthropathy.

Key words

Chemokine, chondrocytes, prostaglandin, MMP.

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Introduction

Chemokines are small, heparin-binding cytokines, characterized by their properties of chemo-attraction. Chemokines constitute distinct subfamilies including C-, CC-, CXC- and CX3C structures, each of which possesses distinct patterns of distribution and expression. Although they were originally suggested to be key players in inflammatory responses, accumulating evidence indicates important contributions of chemokines to cartilage metabolism mediated through articular chondrocytes [reviewed in (1)]. In particular, we and others have demonstrated up-regulated expression of chemokines, such as monocyte chemoattractant protein MCP)-1 (CCL2), RANTES (regulated upon activation, normal T cell expressed and secreted) (CCL5), macrophage inflammatory factor (MIP)-1β (CCL4) or stromal cell-derived factor (SDF)-1 (CXCL12), along with their receptors (CCR/CXCR), in human chondrocytes, suggesting a role for these chemokines in cartilage degeneration in osteoarthritis (OA) and rheumatoid arthritis (RA) (2-6). The induction of matrix metalloproteinases (MMPs) in chondrocytes after chemokine stimulation has been reported by several groups including ours (4-6). In addition, interestingly, a recent report by Mazzetti *et al.* (7) demonstrated that chemokines could modulate chondrocyte phenotype, clearly indicating an as yet unrecognized function of chemokines in chondrocyte metabolism.

Prostaglandins (PGs) are known to play an important role in the pathophysiology of inflammatory joint lesions. PGE_2, the most abundant PG, has been detected at high levels in synovial fluid in arthropathy (8). Chondrocytes express both cyclooxygenase (COX)-2 (9) and prostaglandin E synthase (PGES) (10), both of which are inducible enzymes responsible for PGE_2 production. It has been suggested that PGs present in the vicinity of cartilage play a role in the modulation of collagen or aggrecan turnover, chondrocyte apoptosis (9), expression of MMPs and chondrocyte production of tissue inhibitors of matrix metalloproteases (TIMP) (11,12), as well as in chondrocyte differentiation (13). PGE_2 is known to be induced by stimulation with proinflammatory cytokines such as IL-1 (10), stimuli that also enhance MMPs. However, it is unclear whether the chondrocyte response to a single chemokine includes both PGs and MMPs simultaneously or at a similar level.

To clarify this issue, here we investigated levels of PGE_2 and of MMP-3 induced by chemokines in monolayer cultures of human articular chondrocytes.

Materials and methods

Patients

Articular cartilage specimens were obtained from 4 OA (age 56-84 yrs., F:M 3:1), 5 RA (age 46-72, F:M 3:2) and 4 trauma patients with fracture (age 59-88, F:M 0:4) undergoing total joint replacement at St. Marianna University (Kawasaki) or at Hara Hospital (Tokyo). The patients with traumatic fracture had no history of arthritis and the cartilage obtained showed no macro- or microscopic joint pathology. Thus, the cells isolated from these samples were considered to represent "normal" chondrocytes. All the samples were obtained with informed consent from the patients and the study protocol was approved by the institutional ethical committee.

Chondrocyte isolation

Cartilage was minced and digested at 37°C in Dulbecco's modified Eagle's medium (DMEM, GIBCO BRL, Grand Island, NY, USA) containing 1 mg/ml of collagenase (Sigma-Aldrich Japan, Co., Tokyo, Japan). The released cells were filtered, washed and centrifuged. The pellet was seeded into collagen I-coated culture flasks and cultivated at 37°C in a humidified atmosphere of 5% CO_2. After reaching confluence, the cells were passaged onto 6-cm dishes and further cultivated. Only the first passage cells were used in the following study.

The culture medium used was DMEM containing 10% heat-inactivated fetal calf serum (FCS, GIBCO BRL), 100 units/ml penicillin (GIBCO BRL) and 100 mg/ml streptomycin (GIBCO BRL).

Before applying the chemokines, cells were serum-starved for 24 h in medium with only 0.5% FCS and then stimulated with one of the following chemokines: MCP-1, RANTES, or SDF-1 (R&D Systems, Inc., Minneapolis, MN) at 100 ng/ml for 24 h. The concentration used was determined to be optimal according to our preliminary experiments (data not shown). Supernatants were then collected and stored at -30°C until used. MMP-3 and PGE2 concentrations in the culture supernatants were evaluated by commercially available kits following the manufacturer's instructions (MMP3: Medical & Biological Laboratories Co, Ltd., Nagoya, Japan; R&D; PGE_2: Cayman Chemical Co., Ann Arbor, MI, USA).

Results

Because chondrocytes express chemokine receptors and chemokines exert catabolic effects via the induction of MMPs, we designed the present experiments to compare the induction of another catabolic factor, PGE_2, in addition to MMP-3, from chondrocytes. To this end, we stimulated chondrocytes *in vitro* with chemokines for 24 h, and analyzed the levels of secreted PGE2 comparing to MMP-3 in the culture supernatants.

Figure 1 shows the effect of chemokines on chondrocyte PGE_2 production. The overall mean value of baseline PGE_2 (before stimulation) in the chondrocyte culture supernatants was 57.5 pg/ml (SD ± 14.7); and there was no significant bias between the patient groups (data not shown). As summarized in Figure 1, chemokines potently induced PG production by human articular chondrocytes but only in a subset of samples. Thus, there seem to be "responders" and "non-responders" to chemokine stimulation under these experimental conditions, regardless of the original clinical diagnosis. Samples that responded to one chemokine also tended to respond to another, whereas the remainder failed to respond to any. For example, RA-1 chondrocytes responded well to all chemokines tested, whereas RA-5 did not respond to any, although both samples were obtained from RA patients and cultured and assayed in the same way. In general, "normal" or arthritis-free chondrocytes were less responsive to chemokine stimulation compared to the OA and RA samples. Of the three chemokines tested, only RANTES stimulated a strong response of up to 200% in all patient groups.

We then compared the PG-stimulating

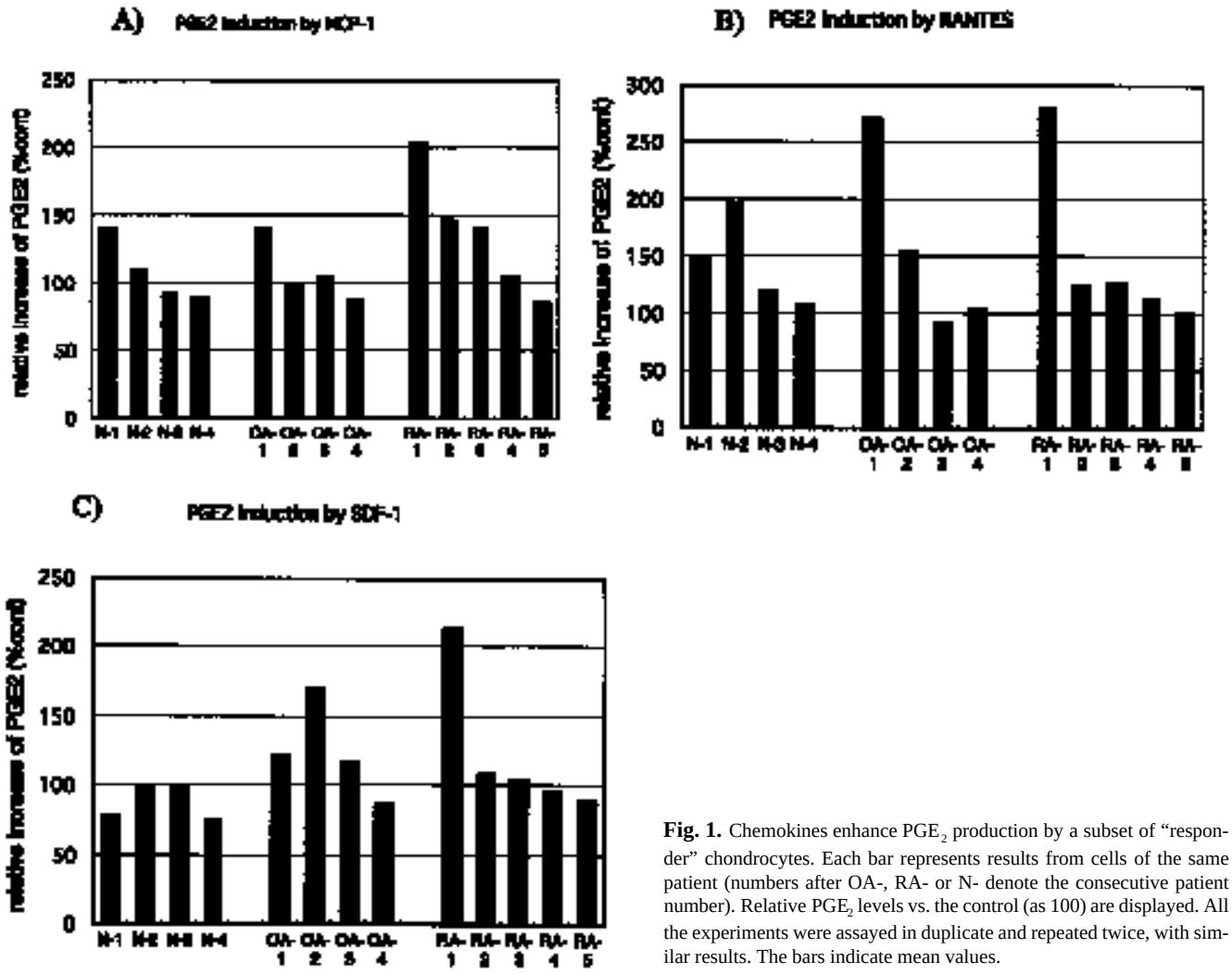

Fig. 1. Chemokines enhance PGE_2 production by a subset of "responder" chondrocytes. Each bar represents results from cells of the same patient (numbers after OA-, RA- or N- denote the consecutive patient number). Relative PGE_2 levels vs. the control (as 100) are displayed. All the experiments were assayed in duplicate and repeated twice, with similar results. The bars indicate mean values.

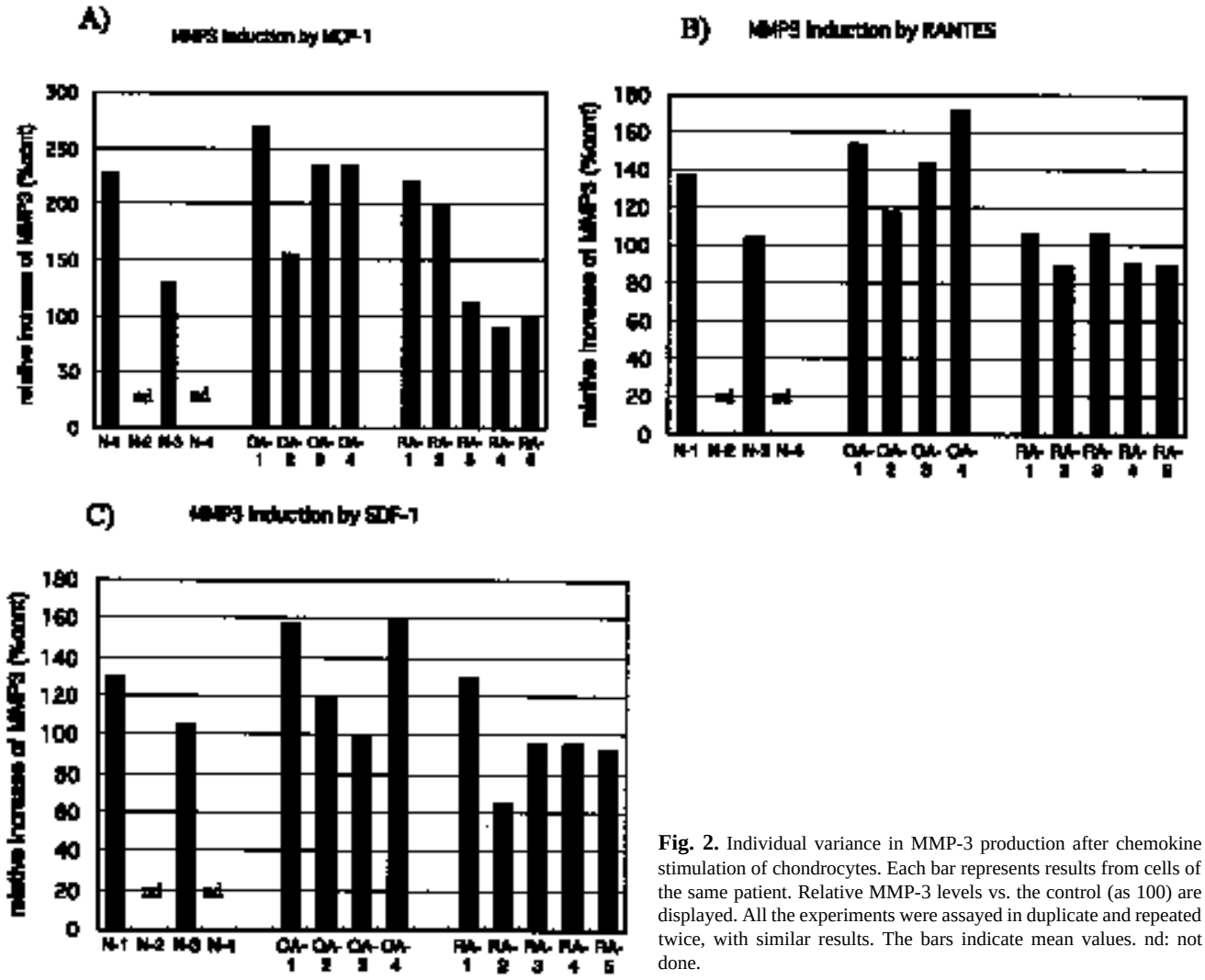

Fig. 2. Individual variance in MMP-3 production after chemokine stimulation of chondrocytes. Each bar represents results from cells of the same patient. Relative MMP-3 levels vs. the control (as 100) are displayed. All the experiments were assayed in duplicate and repeated twice, with similar results. The bars indicate mean values. nd: not done.

capacity of these chemokines with MMP-3 induction in the same samples. As previously reported, chemokines induced MMP-3 production by chondrocytes as shown in Figure 2. Again, it was noted that these responses differed considerably among the chondrocyte samples, with some maintaining almost the same level of MMP-3 or even showing a slight decrease after stimulation. The response pattern in each sample was not necessarily the same for PGE_2 and MMP-3, possibly suggesting distinct signaling pathways, rather than reflecting non-specific chondrocyte activation.

Figure 3 shows representative results of MMP-3 and PGE2 ELISA in two "responder" samples. The absolute values of MMP-3 and PGE2 in culture supernatants varied considerably between samples. Nevertheless, the chemokine response pattern was similar in these samples.

Discussion

Human articular chondrocytes express a wide range of G-protein-coupled receptors that deliver distinct signals to the cells. In particular, the chemokine receptor family is expressed by chondrocytes, and possibly upregulated upon stimulation with various agents (4, 5). In addition to their major participation in inflammatory processes, chemokines are now believed to be important players in cartilage degradation in joint diseases such as RA and OA. However, the precise mechanisms by which the chemokines affect chondrocyte metabolism are still not fully clarified.

Prostaglandins including PGE_2 have been widely investigated with regard to their important contribution to inflammation, and also concerning their influence on cartilage metabolism. Although the effect of PGE_2 may vary depending on the milieu of its action, it is known to be induced in human articular chondrocytes after proinflammatory cytokine stimulation (10), suggesting a role in arthritic inflammation.

Our present study is the first to demonstrate a role for chemokines in the induction of PGE_2, at least in a subset of arthritic and non-arthritic patient samples. Although chondrocyte PGE_2 responses varied between samples, the presence of a signaling pathway by which chemokine stimulation can lead to PGE_2 secretion is apparent. Because MCP-1, RANTES and SDF-1 utilize distinct receptors (CCR2 for MCP-1, CCR-5 (mostly) for RANTES and CXCR4 for SDF-1) (1), the distribution

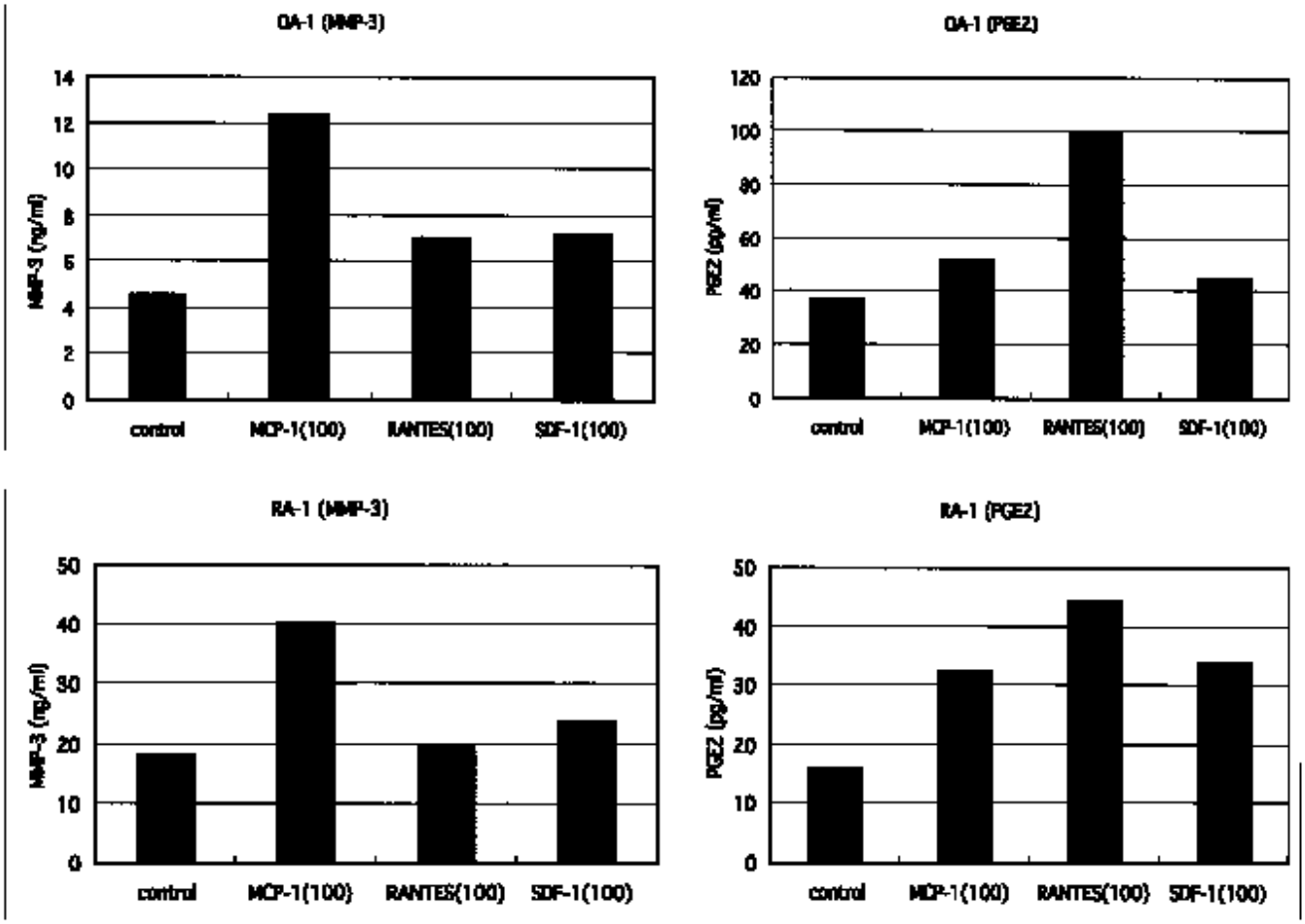

Fig. 3. Induction of MMP-3 and PGE_2: representative results. The absolute values for two representative chondrocyte samples (OA-1 and RA-1) are shown. The bars indicate mean values of ELISA.

pattern of these receptors in chondrocytes will influence the response. Nevertheless, some patients were shown to respond to all these chemokines, suggesting a common pathway encompassing these chemokine-chemokine receptor signaling events. Unfortunately, because of limited cell availability we were unable to compare the expression of chemokine receptors in these samples in a quantitative manner in the present study. This issue requires further investigation. In addition, the nature of the signal transduction pathways remains to be clarified. However, our preliminary data suggest that PGE_2 induction by chemokines could be completely blocked either by p38 or ERK MAP kinase inhibitors (data not shown).

One important conclusion from the present study is that caution must be exercised when considering the role of chemokines in cartilage degradation. Although previous studies demonstrated that chemokines induce MMPs and matrix degradation *in vitro*, the overall effect of chemokines on chondrocyte metabolism might differ in degree between the samples or patients studied. In fact, chemokine/chemokine receptor systems are known to be very complicated; thus there may be compensatory effects for each specific stimulus. In addition, the heterogeneous nature of *in vitro* cultured articular chondrocytes obtained from a single joint is implied. In this regard, using cartilage explant cultures Hardy *et al*. (14) reported that articular cartilage specimens of OA patients produced only low levels of PGE_2 upon IL-1 stimulation; they also observed a large variability of responses in the tissue. Thus, chondrocytes taken from specific lesions (e.g. the surface or a deeper zone of cartilage) might need to be evaluated separately. We agree with the view of Hardy *et al*. (14) that the effect of synovial tissue-derived, but not cartilage-derived chemokines and PGs are crucial when analyzing the *in vivo* effect of catabolic factors, because the absolute values of MMP and PGs produced from chondrocytes would be much lower than from synoviocytes. According to the study by Punzi *et al.*, the level of PGE_2 in RA joint fluid was around 60-100 pg/ml (15), with most of it suggested to be derived from inflamed synovial tissue. Nevertheless, the contribution of chondrocytes to eicosanoid production in joint lesions is worth further investigation, as it might provide a new avenue to establish cartilage-specific anti-inflammatory strategies, especially in degenerative process such as OA, without severe synovitis.

In conclusion, we have demonstrated here that chemokines may enhance prostaglandin production from a subset of human articular chondrocytes.

Understanding the role of inflammatory mediators in cartilage degradation might lead to the development of new strategies to combat arthropathies.

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