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J Dent Res 88(6):557-562, 2009

ABSTRACT

Similarly to humans, healthy, wild-type mice develop osteoarthritis, including of the temporomandibular joint (TMJ), as a result of aging. Pro-inflammatory cytokines, such as IL-1 β , IL-6, and TNF α , are known to contribute to the development of osteoarthritis, whereas TGF β has been associated with articular regeneration. We hypothesized that a balance between IL-1 β and TGF β underlies the development of TMJ osteoarthritis, whereby IL-1 β signaling down-regulates TGF β expression as part of disease pathology. Our studies in wild-type mice, as well as the Col1-IL1 β^{XAT} mouse model of osteoarthritis, demonstrated an inverse correlation between IL-1 β and TGF β expression in the TMJ. IL-1 β etiologically correlated with joint pathology, whereas TGF β expression associated with IL-1 β down-regulation and improvement of articular pathology. Better understanding of the underlying inflammatory processes during disease will potentially enable us to harness inflammation for orofacial tissue regeneration.

KEY WORDS: IL-1 β , TMJ pathology, TGF β .

IL-1 β Inhibits TGF β in the Temporomandibular Joint

INTRODUCTION

Osteoarthritis (OA) is a progressively destructive joint disorder with articular cartilage erosions and fibrillations, as well as clustering and proliferation of articular chondrocytes (Hamerman, 1989; Andriamanalijaona *et al.*, 2003; Clements *et al.*, 2003). The prevalence of OA in the population over the age of 40 is as high as 90% (Frey *et al.*, 1999). Age-related changes are characterized by significant reduction of cellularity, glycosaminoglycan content, and synthesis of collagen (Livne, 1994; Temple *et al.*, 2007). Recent studies have implicated inflammatory processes in OA pathogenesis (Rosen *et al.*, 1997; Fukui *et al.*, 2003; Lai *et al.*, 2006). IL-1 β levels were found to increase during early stages of OA (Pelletier and Martel-Pelletier, 1989), leading to cartilage breakdown and the inhibition of proteoglycan synthesis (Goldring *et al.*, 1996; Martel-Pelletier *et al.*, 1999; Westacott and Sharif, 1999; Scharstuhl *et al.*, 2002; Blaney-Davidson *et al.*, 2005; Lai *et al.*, 2006). OA pathology is also characterized by a hypertrophic response at early disease stages, including articular chondrocyte proliferation and the expression of growth factors as part of an attempted repair process (Andriamanalijaona *et al.*, 2003; Wadhwa *et al.*, 2005). To this end, TGF β promotes the formation of extracellular matrix and stimulates chondrocyte proliferation (Villiger *et al.*, 1993; Blaney-Davidson *et al.*, 2005).

The purpose of the present study was to investigate the relationship of IL-1 β and TGF β in TMJ osteoarthritis. The TMJ differs anatomically from other joints (*i.e.*, knee) in both composition, since it is comprised of fibrocartilage, and function, since it is exposed to limited load-bearing forces, such as mastication (Tanaka *et al.*, 2008). Nevertheless, the TMJ, like the knee and other joints, develops OA with aging (Livne, 1994). However, OA in load-bearing joints appears to develop more rapidly than in the TMJ. By 6 months of age in mice, complete erosion of the articular cartilage was found in the knee, while only early histological osteoarthritic lesions were detected in the TMJ (Wadhwa *et al.*, 2005). These differences between the knee and the TMJ can be partly explained by differences in force magnitude applied to joints, causing a greater amount of articular degeneration (Temple *et al.*, 2007).

We hypothesized that a balance between IL-1 β and TGF β underlies the development of TMJ osteoarthritis, whereby IL-1 β signaling down-regulates TGF β expression as part of disease pathology. To address this hypothesis, we used two mouse models of TMJ osteoarthritis, the first occurring naturally with age in the TMJ of wild-type mice, and the second model in the Col1-IL1 β^{XAT} transgenic mouse (Lai *et al.*, 2006). The Col1-IL1 β^{XAT} transgenic mouse served as the substrate for somatic mosaic analysis, a method for altering the composition of somatic cells in specific areas of the body, hence creating a mosaic of somatic cells. In this transgenic mouse, the pro-collagen I promoter (Col1) drives the expression of the transcriptionally dormant gene Col1-loxP-STOP-loxP-IL1 β -IRES-lacZ-pA (Col1-IL1 β^{XAT}). The Col1-IL1 β^{XAT}

DOI: 10.1177/0022034509336823

Received June 16, 2008; Last revision January 14, 2009;
Accepted January 30, 2009

Figure 1. Wild-type mice spontaneously develop arthritis-like TMJ pathology. **(A)** Two-month-old C57Bl6 wild-type mice showed normal TMJ histology (score = 1). However, **(B)** when they were 6 mos of age, we observed histopathological changes, including loss of normal cyto-architecture, decreased articular cellularity, and chondrocyte cloning (score = 2). **(C)** The aforementioned joint pathology was partially ameliorated by 12 mos of age (score = 1). **(D)** Abrogation of IL-1 signaling following targeted deletion of the type I receptor for IL-1 (IL1RI^{-/-} knockout mice) normalized joint histology (score = 1). **(E)** IL-1β expression was evaluated by immunohistochemistry and was found to be low in 2-month-old mice, but **(F)** was found to be increased at 6 mos of age. Furthermore, **(G)** IL-1β immunoreactivity was found to be reduced at 12 mos of age. **(H)** Deletion of the IL1RI receptor did not affect IL-1β expression in 6-month-old mice. Moreover, **(I)** we observed elevated TGFβ expression at 2 mos of age, which **(J)** was reduced in 6-month-old mice. Moreover, **(K)** TGFβ immunoreactivity was restored at 12 mos of age. **(L)** Deletion of the IL1RI receptor restored the aforementioned TGFβ down-regulation in 6-month-old mice. Bar = 100 μm.

is an **eXcisionally Activated Transgene (XAT)** based on the Cre/loxP molecular genetic method that utilizes a germline-transmitted recombinational substrate containing a dormant transcription unit coupled to somatic gene transfer of Cre recombinase to activate the gene of interest (Lai *et al.*, 2006; Kyrkanides *et al.*, 2007; Fiorentino *et al.*, 2008). Delivery of Cre recombinase was accomplished *via* the injection of the defective, recombinant feline immunodeficiency viral vector, FIV(Cre), into the TMJs of 2-month-old mice (Lai *et al.*, 2006; Kyrkanides *et al.*, 2007; Fiorentino *et al.*, 2008). Therefore, we evaluated changes in IL-1β and TGFβ expression in the TMJs of WT and Col1-IL1β^{XAT} mice as they relate to the development of OA with aging.

MATERIALS & METHODS

Animal Studies

All animal procedures were approved by the Institutional Animal Care and Use Committee prior to the initiation of the study. Wild-type mice, IL1RI^{-/-} knockout mice (Jax Laboratories, Manassas, VA, USA) and Col1-IL1β^{XAT} transgenic mice (Lai *et al.*, 2006) were used on a pure C57/Bl6 strain background. We

accomplished deletion of the IL-1β type I receptor (IL1RI) in the Col1-IL1β^{XAT} transgenic mice by crossing the IL1RI^{-/-} knockout mice into Col1-IL1β^{XAT} transgenic mice. Two-month-old Col1-IL1β^{XAT} transgenic mice were injected with 10 μL of FIV(Cre) in the right and left TMJs (a total of 1 × 10⁶ infectious particles) by means of a 271/2 gauge needle under surgical anesthesia (ketamine 40 mg/Kg IP). Additional groups of Col1-IL1β^{XAT} mice received TMJ injections of the control vector FIV(gfp), encoding for the reporter gene green fluorescent protein at the same titer as FIV(Cre), controlling for the viral injection. Additional mice received an equal volume of saline controlling for the injection procedure. The mice were killed at 2 and 6 mos after injections, and their skulls were harvested and immersed in 10% formalin solution for fixation. In total, 26 Col1-IL1β^{XAT} transgenic mice (13 males and 13 females) were used in this study. Nine Col1-IL1β^{XAT} transgenic mice received FIV(Cre) injection (5 mice were killed at 2 mos and 4 mice at 6 mos after viral transduction), and 9 received FIV(gfp) in their TMJs (5 mice were killed at 2 mos and 4 mice at 6 mos after viral transduction). In addition, 8 (5 males, 3 females) Col1-IL1β^{XAT};IL1RI^{-/-} compound mice were injected with FIV(Cre) in the TMJ (4 mice were killed at 2 mos and 4 mice at 6 mos after viral transduction). In

addition, 13 wild-type mice (7 males, 6 females) were used in this study, with 5 mice killed at 2 mos, 4 mice at 6 mos, and 4 mice at 12 mos of age. An additional 13 IL1RI^{-/-} knockout mice (5 males, 8 females) were also included in the study: 5 mice were killed at 2 mos, 4 mice at 6 mos, and 4 mice at 12 mos of age.

Histology

Following fixation in 10% formalin, the mouse heads were dissected, de-fleshed, and decalcified by immersion in an EDTA solution for 7 days at 4°C under constant agitation. The TMJs were processed in a RHS-1 microwave tissue processor (Hacker Instruments, Winnsboro, SC, USA), and then embedded in paraffin, cut into 5- μ m-thick sections, and collected on glass slides. Each animal contributed 2 joints (left and right TMJs), and each joint was represented once in our analysis. The sections represented mid-sagittal portions of the TMJ. Histopathology was evaluated by Alcian blue-orange G histochemistry on a scale of 0-4 as previously described (Wilhelmi and Faust, 1976; Lai *et al.*, 2006; Kyrkanides *et al.*, 2007; Fiorentino *et al.*, 2008). Briefly, this scale is defined as: 0 = no apparent changes; 1 = superficial erosions, articular fibrillations, or striations; 2 = injuries limited to uncalcified cartilage; 3 = defects extending into calcified cartilage; and 4 = defects extending into calcified bone. We assessed articular chondrocyte cloning by counting the number of lacunae containing 2 or more articular chondrocytes under 40X magnification and subsequently averaged them *per* TMJ section.

Immunohistochemical (IHC) analysis was performed for several antigens with the antibodies described below. Sections were rehydrated in PBS for 60 min and bleached in 3% H₂O₂ for 15 min. Tissues were blocked with appropriate normal serum (4% solution in PBS) followed by overnight incubation in primary antibody solution at room temperature. The tissue was then rinsed in PBS, blocked by appropriate serum (4% solution in PBS), and incubated in the appropriate biotinylated secondary antibody solution for 90 min, followed by PBS rinse and incubation in ABC solution (Vector Laboratories, Burlingame, CA, USA) for 90 min. Immunoreactivity was developed with Nickel-DAB. The histological sections were evaluated by light microscopy with an Olympus BX51 microscope. Microphotographs were captured by means of a Spot CCD digital camera attached to the microscope. The antibodies were a rabbit anti-murine IL-1 β polyclonal antibody (Antigenix America, Huntington Station, NY, USA) at 0.2 μ g/mL; rabbit anti-human (mature) IL-1 β polyclonal antibody (Abcam, Cambridge, MA, USA) used at 1 μ g/mL; and rabbit anti-TGF β polyclonal antibody (Santa Cruz

Figure 2. IL-1 β expression is associated with articular pathology and TGF β down-regulation in the TMJ. **(A)** TMJ pathology was evaluated by a 1-to-4 scale introduced by Wilhelmi and Faust (1976) in the TMJs of wild-type C57/Bl mice over time. **(B)** We used articular cloning as an additional method of assessing arthritis-like changes in the TMJ. **(C)** The levels of IL-1 β and TGF β expression, presented as numbers of immunoreactive cells staining positive for these two antigens, are presented. Please note the inverse relationship of IL-1 β and TGF β expression levels in the TMJ, which changes with time. Furthermore, note how increased IL-1 β temporally correlates with higher articular pathology levels in 6-month-old wild-type mice, whereas higher TGF β levels temporally correlate with low articular pathology levels. In total, 26 Col1-IL1 β ^{XAT} transgenic mice (13 males and 13 females) were used in this study. Nine Col1-IL1 β ^{XAT} transgenic mice received FIV(Cre) injection (5 mice were killed at 2 mos and 4 mice at 6 mos after viral transduction), and 9 received FIV(gfp) in their TMJ (5 mice were killed at 2 mos and 4 mice at 6 mos after viral transduction). In addition, 8 (5 males, 3 females) Col1-IL1 β ^{XAT};IL1RI^{-/-} compound mice were injected with FIV(Cre) in the TMJ (4 mice were killed at 2 mos and 4 mice at 6 mos after viral transduction). In addition, 13 wild-type mice (7 males, 6 females) were used in this study, with 5 mice killed at 2 mos, 4 mice at 6 mos, and 4 mice at 12 mos of age. An additional 13 IL1RI^{-/-} knockout mice (5 males, 8 females) were also included in the study: 5 mice were killed at 2 mos, 4 mice at 6 mos, and 4 mice at 12 mos of age. Mean ($\pm$ SEM). * p < 0.05.

Biotechnology, Santa Cruz, CA, USA) at 1 μ g/mL. Control conditions included tissue sections that were processed by immunohistochemistry in the absence of a primary antibody. The number of cells staining positive after immunohistochemistry were counted in each TMJ section under 40X by one investigator (SK). All tissue sections were processed simultaneously for every antigen.

Statistical Analysis

Data were compared by analysis of variance and Tukey's *post hoc* tests. P values less than 0.05 were considered significant.

RESULTS

Wild-type Mice Spontaneously Develop TMJ Arthritis with Age

Wild-type mice were evaluated at 2, 6, and 12 mos of age for changes in TMJ histology by Alcian blue-orange G histochemistry. Overall, 2-month-old wild-type mice displayed normal TMJ histology (Fig. 1A). However, by 6 mos of age, the mice displayed joint pathology in the TMJ (Fig. 1B), which was spontaneously attenuated by 12 mos of age (Fig. 1C). We assessed the expression of IL-1 β in the TMJ and found a significant increase in the number of IL1 β -positive cells in the 6-month-old animals (Figs. 1F, 2C), occurring concurrently with the highest degree of TMJ pathology (Figs. 2A, 2B, 2C). Conversely, IL-1 β expression decreased to near-normal levels in 12-month-old mice (Figs. 1G, 2C), when articular pathology spontaneously

Figure 3. IL-1 β over-expression in the TMJ induces arthritis-like pathology and decreases TGF β expression. Injection of FIV(Cre) into the TMJs of Col1-IL1 β^{XAT} transgenic mice resulted (**A**) in the development of arthritis-like pathology, including normal cyto-architecture, decreased articular cellularity, and chondrocyte cloning (score = 3). With time, however, (**B**) the observed joint pathology ameliorated spontaneously (score = 1). In addition, (**C**) ablation of IL-1 β signaling resulted in the prevention of joint pathology development, as observed in Col1-IL1 $\beta^{XAT};IL1RI^{-/-}$ compound mice (score = 1). (**D**) Injection of the control vector FIV(gfp), encoding for the reporter gene green fluorescent protein, did not induce any articular changes in the Col1-IL1 β^{XAT} transgenic mice (score = 1). IL-1 β immunoreactivity correlated with the development of articular pathology, (**E**) was increased at the 2-month time-point, and (**F**) decreased at 6 mos due to transgene silencing. (**G**) Deletion of the IL1RI receptor did not alter IL-1 β expression by articular chondrocytes. (**H**) Injection of the control vector FIV(gfp) did not IL-1 β over-expression in the TMJ. Moreover, we evaluated TGF β immunoreactivity in the TMJ during the aforementioned conditions and found (**I**) that TGF β expression was decreased following the injection of FIV(Cre) into the TMJ of Col1-IL1 β^{XAT} transgenic mice. Conversely, (**J**) TGF β expression was normalized in TMJs experiencing transgene silencing 6 mos after FIV(Cre) injection into the TMJ of Col1-IL1 β^{XAT} transgenic mice. (**K**) TGF β expression was also restored in Col1-IL1 $\beta^{XAT};IL1RI^{-/-}$ compound mice. (**L**) Col1-IL1 β^{XAT} transgenic mice injected with the control vector FIV(gfp) showed normal levels of TGF β immunoreactivity in the TMJ.

ameliorated (Figs. 1C, 2C). Abrogation of IL-1 β signaling in IL1RI $^{-/-}$ mice prevented joint pathology (Fig. 1D).

We then assessed TGF β expression in the TMJ, since this growth factor was previously associated with joint regeneration. We found that TGF β levels changed inversely with IL-1 β (Fig. 2C). We observed high levels of TGF β expression in 2- (Fig. 1I) and 12-month-old mice (Fig. 1K), concurrently with low IL-1 β expression (Figs. 1E and 1G, respectively). Conversely, low levels of TGF β expression were observed in 6-month-old mice (Fig. 1J), concurrently with high levels of IL-1 β expression (Fig. 1F) and an increased degree of joint histopathology (Fig. 1B).

IL-1 β Signaling Modulates TGF β Expression in the TMJ

TGF β appears to have a steady-state expression in healthy TMJ tissues (Figs. 1I, 1K), corresponding to low IL-1 β levels of expression (Figs. 1E, 1G). However, increased IL-1 β expression, as observed in 6-month-old mice (Figs. 1F, 2C), corresponded to TGF β down-regulation (Figs. 1J, 2C).

Furthermore, deletion of the IL1RI receptor abrogated the negative regulatory effects of IL-1 β on TGF β expression (Figs. 1H, 1L). Taken together, the aforementioned observations suggest IL-1 β inhibiting TGF β expression through the IL1RI receptor.

To investigate further the relationship of IL-1 β and TGF β *in vivo*, we over-expressed IL-1 β in the TMJs of adult Col1-IL1 β^{XAT} mice and evaluated TGF β expression and TMJ histopathology. Two months following the induction of IL-1 β , we observed TMJ osteoarthritis (Fig. 3A) concurrently with TGF β down-regulation (Fig. 3I). Six months following IL-1 β induction in Col1-IL1 β^{XAT} mice, we observed amelioration of the joint pathology (Fig. 3B). This occurred spontaneously after IL-1 β down-regulation (Fig. 3F), apparently due to transgene silencing, which also allowed for homeostatic up-regulation of TGF β (Fig. 3J). Hence, IL-1 β signaling negatively regulated TGF β signaling during the development of arthritis (Fig. 4). This was also seen following IL1RI receptor deletion, since TGF β expression and TMJ histology were restored in Col1-IL1 $\beta^{XAT};IL1RI^{-/-}$ mice.

DISCUSSION

Analysis of the aforementioned data demonstrates spontaneous development of TMJ osteoarthritis in wild-type mice with age, in accordance with reports from previous studies (Wadhwa *et al.*, 2005). Similarly, human joints also develop osteoarthritis as a result of aging (DeBont *et al.*, 1985a,b; Ribeiro *et al.*, 1997; Nebbe and Major, 2000). The role of pro-inflammatory cytokines (IL-1 β , TNF α , IL-6) in the development of OA in mammals has been previously suggested (Krauss and Shu, 1997; Goldring and Goldring, 2004). Smith *et al.* (1997) examined the levels of IL-1 α and IL-1 β in synovial membranes from persons with OA and suggested that chronic inflammatory changes induced by IL-1 β are characteristic of early OA. Moos *et al.* (1999) demonstrated that IL-1 β was up-regulated in cartilage harvested from knee or hip joints derived from persons with OA compared with healthy individuals. Webb *et al.* (1998) demonstrated that OA synovium supernatants contained higher concentrations of interleukin-1 β than normal synovial supernatants. Furthermore, IL-1 β levels were found to be increased in synovial tissue harvested from persons suffering from TMJ disorders when compared with healthy individuals (Ogura *et al.*, 2002; Suzuki *et al.*, 2002).

Early stages of OA are characterized by activation and proliferation of chondrocytes, which start forming clusters and actively produce matrix molecules, including collagen II (Mankin, 1974; Silberman and Livne, 1979; Brandt, 1991; Guerne *et al.*, 1995; Rosen *et al.*, 1997; Barbero *et al.*, 2004). This hypertrophic reaction has been considered to be an attempt at articular repair. TGF β has been previously proposed as one of the best candidate genes to stimulate cartilage repair (Rdini, 1993; Pujol *et al.*, 1994, 1999; Hunziker, 2001). There are several ways by which TGF β can be involved in articular regeneration. First, TGF β has profound effects on the differentiation and proliferation of chondrocytes (Villiger *et al.*, 1993; Hering *et al.*, 2001; Bira *et al.*, 2005). Second, proteoglycan synthesis is enhanced and proteases are down-regulated (Serra *et al.*, 1997; Scharstuhl *et al.*, 2002). A significant down-regulation of TGF β was observed in aged mice compared with young ones (Serra *et al.*, 1997; Scharstuhl *et al.*, 2002; Xiang *et al.*, 2006). In our studies, IL-1 β up-regulation in the TMJ corresponded with TGF β down-regulation and increased levels of joint pathology. In addition, accelerated over-expression of IL-1 β in the adult mouse TMJ resulted in the inhibition of TGF β expression, which was prevented after abrogation of IL1RI signaling.

In conclusion, our studies in wild-type mice, as well as the Col1-IL1 β^{xAT} mouse model of osteoarthritis, demonstrated an inverse correlation between IL-1 β and TGF β expression in the TMJ. IL-1 β correlated etiologically with joint pathology, whereas TGF β expression was associated with IL-1 β down-regulation and improvement of articular pathology. Better understanding of the underlying inflammatory processes during disease will potentially enable us to harness inflammation for orofacial tissue regeneration.

ACKNOWLEDGMENTS

This work was funded by grants DE017765 and AR055035 from the National Institutes of Health.

Figure 4. Spontaneous amelioration of joint pathology following IL-1 β over-expression in the TMJ of Col1-IL1 β^{xAT} transgenic mice. Transgene activation and IL-1 β induction in the TMJ of the Col1-IL1 β^{xAT} mouse model resulted in the development of joint pathology 2 mos after viral transduction. At 6 mos after viral transduction, however, we observed a spontaneous amelioration of the joint pathology, concomitantly with a reduction in human hIL-1 β expression in the TMJ, due to apparent transgene silencing. At the same time, the level of TGF β expression was significantly increased at the 6-month time-point compared with the 2-month time-point ($P < 0.001$). Deletion of the IL1RI receptor in this mouse model prevented the development of joint pathology, despite the induction of transgenic IL-1 β . Abrogation of IL-1 signaling allowed for increased TGF β levels of expression. In total, 26 Col1-IL1 β^{xAT} transgenic mice (13 males and 13 females) were used in this study. Nine Col1-IL1 β^{xAT} transgenic mice received FIV(Cre) injection (5 mice were killed at 2 mos and 4 mice at 6 mos after viral transduction), and 9 received FIV(gfp) in their TMJs (5 mice were killed at 2 mos and 4 mice at 6 mos after viral transduction). In addition, 8 (5 males, 3 females) Col1-IL1 $\beta^{xAT};$ IL1RI^{-/-} compound mice were injected with FIV(Cre) in the TMJ (4 mice were killed at 2 mos and 4 mice at 6 mos after viral transduction). In addition, 13 wild-type mice (7 males, 6 females) were used in this study, with 5 mice killed at 2 mos, 4 mice at 6 mos, and 4 mice at 12 mos of age. An additional 13 IL1RI^{-/-} knockout mice (5 males, 8 females) were also included in the study: 5 mice were killed at 2 mos, 4 mice at 6 mos, and 4 mice at 12 mos of age. Mean ($\pm$ SEM).

REFERENCES

- Andriamanalijaona R, Felisaz N, Kim SJ, King-Jones K, Lehmann M, Pujol JP, *et al.* (2003). Mediation of IL-1 β -induced TGF β 1 expression by Activator Protein 4 Transcription Factor in primary cultures of bovine articular chondrocytes. *Arthritis Rheum* 48:1569-1581.
- Barbero A, Grogan S, Schafer D, Heberer M, Mainil-Varlet P, Martin I (2004). Age related changes in human articular chondrocyte yield, proliferation and post-expansion chondrogenic capacity. *Osteoarthritis Cartilage* 12:476-484.
- Bira Y, Tani K, Nishioka Y, Miyata J, Sato K, Hayashi A, *et al.* (2005). Transforming growth factor beta stimulates rheumatoid synovial fibroblasts via the type II receptor. *Mol Rheumatol* 15:108-113.

- Blaney-Davidson EN, Scharstuhl A, Vitters EL, van der Kraan PM, van den Berg WB (2005). Reduced TGF β signaling in cartilage of old mice: role in impaired repair capacity. *Arthritis Res Ther* 7:1338-1347.
- Brandt KD (1991). Animal models: insights into osteoarthritis (OA) provided by the cruciate-deficient dog. *Br J Rheumatol* 30(Suppl 1):5-9.
- Clements KM, Price JS, Mason RM, Chambers MG, Visco DM, Poole AR (2003). Gene deletion of either IL-1 β , IL-1 β -converting enzyme, inducible nitric oxide synthase, or stromelysin 1 accelerates the development of knee osteoarthritis in mice after surgical transection of the medial collateral ligament and partial medial meniscectomy. *Arthritis Rheum* 48:3452-3463.
- De Bont LG, Blankestijn J, Panders AK, Vermey A (1985a). Unilateral condylar hyperplasia combined with synovial chondromatosis of the temporomandibular joint. *J Maxillofac Surg* 13:32-36.
- De Bont LG, Boering G, Liem RS, Havinga P (1985b). Osteoarthritis of the temporomandibular joint: a light microscopic and scanning electron microscopic study of the articular cartilage of the mandibular condyle. *J Oral Maxillofac Surg* 43:481-488.
- Fiorentino PM, Tallents RH, Miller JH, Brouxhon SM, Centola G, O'Banion MK, *et al.* (2008). Spinal IL-1 β in a mouse model of arthritis and joint pain. *Arthritis Rheum* 58:3100-3109.
- Frey RJ, Olendorf D, Jeryn C, Boyden K (1999). The Gale encyclopedia of medicine: osteoarthritis. Farmington Hills, MI: Gale Research Group.
- Fukui N, Zhu Y, Maloney W, Clohisy J, Sandell LJ (2003). Stimulation of BMP-2 expression by pro-inflammatory cytokines IL-1 and TNF α in normal and osteoarthritic chondrocytes. *J Bone Joint Surg Am* 85(Suppl 3):59-66.
- Goldring MB, Suen LF, Yamin R, Lai WF (1996). Regulation of collagen gene expression by prostaglandins and interleukin-1 β in cultured chondrocytes and fibroblasts. *Am J Ther* 3:9-16.
- Goldring SR, Goldring MB (2004). The role of cytokines in cartilage matrix degeneration in osteoarthritis. *Clin Orthop Relat Res* 437:27-36.
- Guerne PA, Blanco F, Kaelin A, Desgeorges A, Lotz M (1995). Growth factor responsiveness of human articular chondrocytes in aging and development. *Arthritis Rheum* 38:960-968.
- Hamerman D (1989). The biology of osteoarthritis. *N Engl J Med* 320:1322-1330.
- Hering S, Isken E, Knabbe C, Janott J, Jost C, Pommer A, *et al.* (2001). TGF β 1 and 2 mRNA and protein expression in human bone samples. *Exp Clin Endocrinol Diabetes* 109:217-226.
- Hunziker EB (2001). Growth-factor-induced healing of partial-thickness defects in adult articular cartilage. *Osteoarthritis Cartilage* 9:22-32.
- Krauss JC, Shu S (1997). Secretion of biologically active superantigens by mammalian cells. *J Hematother* 6:41-51.
- Kyrkanides S, Fiorentino PM, Gan Y, Lai Y-C, Shaftel SS, Puzas JE, *et al.* (2007). Intra-articular μ -opioid receptor induction ameliorates arthritic pain and joint pathology. *Arthritis Rheum* 56:2038-2048.
- Lai Y, Shaftel SS, Miller JH, Tallents RH, Chang Y, Pinkert CA, *et al.* (2006). Intraarticular induction of interleukin-1 β expression in the adult mouse, with resultant temporomandibular joint pathologic changes, dysfunction, and pain. *Arthritis Rheum* 65:1184-1197.
- Livne E (1994). Matrix synthesis in mandibular condylar cartilage of aging mice. *Osteoarthritis Cartilage* 2:187-197.
- Mankin HJ (1974). The reaction of articular cartilage to injury and osteoarthritis. *N Engl J Med* 291:1335-1340.
- Martel-Pelletier J, Alaaeddine N, Pelletier JP (1999). Cytokines and their role in the pathophysiology of osteoarthritis. *Front Biosci* 4:694-703.
- Moos V, Fickert S, Müller B, Weber U, Sieper J (1999). Immunohistological analysis of cytokine expression in human osteoarthritic and healthy cartilage. *J Rheumatol* 26:870-879.
- Nebbe B, Major PW (2000). Prevalence of TMJ disc displacement in a pre-orthodontic adolescent sample. *Angle Orthod* 70:454-463.
- Ogura N, Tobe M, Sakamaki H, Kujiraoka H, Akiba M, Abiko Y, *et al.* (2002). Interleukin-1 β induces interleukin-6 mRNA expression and protein production in synovial cells from human temporomandibular joint. *J Oral Pathol Med* 31:353-360.
- Pujol JP (1999). TGF- β and osteoarthritis: in vivo veritas? *Osteoarthritis Cartilage* 7:439-440.
- Rédini F, Mauviel A, Pronost S, Loyau G, Pujol JP (1993). Transforming growth factor beta exerts opposite effects from interleukin-1 beta on cultured rabbit articular chondrocytes through reduction of interleukin-1 receptor expression. *Arthritis Rheum* 36:44-50.
- Ribeiro RF, Tallents RH, Katzberg RW, Murphy WC, Moss ME, Magalhaes AC, *et al.* (1997). The prevalence of disc displacement in symptomatic and asymptomatic volunteers aged 6 to 25 years. *J Orofac Pain* 11:37-47.
- Rosen F, McCabe G, Quach J, Solan J, Terkeltaub R, Seegmiller J, *et al.* (1997). Differential effects of aging on human chondrocyte responses to transforming growth factor beta: increased pyrophosphate production and decreased cell proliferation. *Arthritis Rheum* 40:1275-1281.
- Scharstuhl A, van Beuningen HM, Vitters EL, van der Kraan PM, van den Berg WB (2002). Loss of TGF counteraction on IL-1 mediated effects in cartilage of old mice. *Ann Rheum Dis* 61:1095-1098.
- Serra R, Johnson M, Filvaroff EH, LaBorde J, Sheehan DM, Derynck R, *et al.* (1997). Expression of a truncated, kinases-defective TGF- β type II receptor in mouse skeletal tissue promotes terminal chondrocyte differentiation and osteoarthritis. *J Cell Biol* 139:541-552.
- Silbermann M, Livne E (1979). Age-related degenerative changes in the mouse mandibular joint. *J Anat* 129(Pt 3):507-520.
- Smith MD, Triantafyllou S, Parker A, Youssef PP, Coleman M (1997). Synovial membrane inflammation and cytokine production in patients with early osteoarthritis. *J Rheumatol* 24:365-371.
- Suzuki T, Segami N, Nishimura M, Nojima T (2002). Co-expression of interleukin-1 β and tumor necrosis factor alpha in synovial tissues and synovial fluids of temporomandibular joint with internal derangement: comparison with histological grading of synovial inflammation. *J Oral Pathol Med* 31:549-557.
- Tanaka E, Detamore MS, Mercuri LG (2008). Degenerative disorders of the temporomandibular joint: etiology, diagnosis, and treatment. *J Dent Res* 87:296-307.
- Temple MM, Bae WC, Chen MQ, Lotz M, Amiel D, Coutts RD, *et al.* (2007). Age- and site-associated biomechanical weakening of human articular cartilage of the femoral condyle. *Osteoarthritis Cartilage* 15:1042-1052.
- Villiger PM, Kusari AB, ten Dijke P, Lotz M (1993). IL-1 β and IL-6 selectively induce TGF β isoforms in human articular chondrocytes. *J Immunol* 151:3337-3344.
- Wadhwa S, Embree MC, Kilts T, Young MF, Ameye LG (2005). Accelerated osteoarthritis in the TMJ of biglycan/fibromodulin double-deficient mice. *Osteoarthritis Cartilage* 13:817-827.
- Webb GR, Westacott CI, Elson CJ (1998). Osteoarthritic synovial fluid and synovium supernatants up-regulate tumor necrosis factor receptors on human articular chondrocytes. *Osteoarthritis Cartilage* 6:167-176.
- Westacott CI, Sharif M (1999). Cytokines in osteoarthritis: mediators or markers of joint destruction? *Semin Arthritis Rheum* 25:254-272.
- Wilhelmi G, Faust R (1976). Suitability of the C57 black mouse as an experimental animal for the study of skeletal changes due to aging, with special reference to osteoarthrosis and its response to tribenoside. *Pharmacology* 14:289-296.
- Xiang Y, Masuko-Hongo M, Sekine T, Nakamura H, Yudoh K, Nishioka K, *et al.* (2006). Expression of proteinase-activated receptors (PAR)-2 in articular chondrocytes is modulated by IL-1 β , TNF- α and TGF- β . *Osteoarthritis Cartilage* 14:1163-1173.