

Biology and pathology of the temporomandibular joint: Is there an animal model to study this affliction?

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Introduction

Temporomandibular joint disorders (TMJD) are characterized by joint degeneration and facial pain. Most often this leads to a significant amount of disability¹⁻³. Histological as well as arthroscopic studies have documented that, at the early stages of the disorders, the tissues of the joint undergo a change resembling osteoarthritis. At very late stages, the joint can become inflamed and the synovitis and bone erosion can resemble an inflammatory arthritis⁴. Unfortunately, at the present time there are no good models that can be used for the analysis of this type of joint destruction in TMJ arthritides. The discussion in this report describes the features and pathology of TMJ disorders and puts forward a potential model that may be amenable for investigation of novel treatments.

TMJ tissues and regulators

Five main tissue types are involved in the normal and pathobiological function of the TMJ. They are articular cartilage, the temporomandibular joint disc, synovial tissue, bone and neurological innervations. All of these tissue systems are affected to some degree by pro-inflammatory cytokines. The recognized key cytokines (and enzyme systems) are TNF α L-1, IL-6 and cyclooxygenase-2. TNF α is a critical modulator of tissue function and is believed to be one of the most important members of the inflammatory cascade. It would be a critical target in ameliorating the progressive degeneration of joint tissues. IL-1 is a ubiquitous regulator of both connective tissue matrix degradation and

bone turnover especially through mechanisms involving matrix metalloproteinases⁵⁻⁸. Thus, evaluation of this cytokine should provide a substantial amount of information on the fate of structural tissues within the joint. IL-6 plays a key role in bone resorption. Elevated levels of this interleukin stimulate osteoclastogenesis as well as bone resorption activity. IL-6 is especially important at sites of inflammation^{9,10}. Quantitation of the levels of this cytokine will provide information on the mechanism of condylar bone resorption. Cyclooxygenase-2 catalyzes the formation of prostaglandins at sites of tissue destruction and inflammation. It is a key regulator of osteoblast activity and is involved in bone repair and the response of tissues to pathological challenges such as inflammation and cancer^{11,12}. This enzyme has never been evaluated in TMJ disorders and could provide important clues as to the progression of the diseases.

Matrix metalloproteinases 3, 9 and 13 are pivotal regulators of collagen destruction in cartilage and bone. MMPs 3 and 9 have been shown to be the most important regulated enzymes of cartilage and connective tissue in pathologies ranging from arthritis to cancer¹³⁻¹⁹. MMP13 is a newly discovered matrix metalloproteinase that is emerging as an important mediator of cartilage and bone development.

A mouse model for TMJ arthritis

All of the tissues described above are available for analysis in the TNF α transgenic mouse, a potential small animal model for the study of joint arthritides.

Over the last two decades several different animal models of arthritis have been developed, including adjuvant arthritis, streptococcal cell-wall arthritis, and antigen-induced arthritis. These models are very informative; however, due to their complexities, the precise mechanism of disease is still unknown. For the purposes of TMJ disorders, the human TNF α transgenic mouse (hTNF α -Tg) may be an ideal model. George Kollias' laboratory made this simple animal model of arthritis by generating mice with a human TNF α transgene in which the AU rich 3' untranslated region (UT)

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region (which shortens mRNA half life) was replaced by the stable β globin 3' UT, resulting in a chronic low level over expression of TNF α ²⁰. This transgene engenders a multi-joint arthritis in the animal. We have recently shown that one of the involved joints is the TMJ. The fact that anti-TNF α therapy prevents development of the disease in the knee suggest a primary role of TNF α in the genesis of the disease. However, it has never been shown that this type of therapy would be effective in the jaw joint.

Macroscopic pathology in this animal model includes swelling of joints at six to eight weeks of age. At 10 weeks of age, some mice begin to suffer loss of movement in their hind legs. Between 10 and 12 weeks the animals progress rapidly toward immobility characterized by synovial hypertrophy, bone resorption and articular cartilage degradation. Joint subluxation can occur after 16 weeks. Progressive weight loss after 12 weeks is a common feature in these mice. The histopathology of the TMJ in this animal indicates that by 12 weeks there is evidence of chondrocyte cloning, bone resorption, cytokine and MMP expression and synovial hypertrophy²¹.

Two hTNF α transgenic mouse lines are in use. They are the 3647 and the 147 lines. The 3647 line is described above. This mouse contains one copy of the transgene and develops a milder, graded form of arthritis²². The mice live for more than one year. In the 197 line, which contains 5 copies of the transgene, the arthritis is more severe with an accelerated onset between 3-5 weeks. The progression of TMJ arthritis in the 3647 line is more similar to the human disease. The staging for different cellular pathologies is more discrete and the inflammation is less severe.

We recognize that the initiating event for TMJ arthritis in the 3647 mouse line is not the same as most variants of human TMJ disorders. However, the cartilage, disc, bone and synovial destruction in this model does match what occurs in the human. If the goal in experimental studies is to better understand the cell behavior in the key structural tissues relating to their destruction, then the initiating arthritic stimulus may be less relevant. That is, articular cloning, MMP activity, osteoclast formation, cytokine production, pain, etc. are manifestations in the tissues that are temporally downstream of the initiating arthritic event. Experiments to characterize these effects and determine if they can be halted or reversed will provide valuable information. In fact, if any novel therapeutic protocols are successful, it will be in the face of a continued chronic stimulus for the arthritis. Thus, it could be argued that they may be effective in patients that have progressed past the early onset of TMJ disorders.

Jaw strength, pain and the TMJ

Patients presenting with orofacial pain from TMJ disorders have been characterized as having decreased bite and chewing forces and limited jaw opening. For example, reduced grip strength²³, sustained jaw closing pain²⁴ and reduced bite strength²⁵ have all been reported in patients with muscle pain. The reduction in muscle force exertion associated with myal-

gia has been suggested to be due to reduced activity of agonist muscles and increased activity of antagonist muscles²⁵⁻²⁹. Keil et al.³⁰ have demonstrated that forelimb grip force reduction is a behavioral index of hyperalgesia in the carrageenan model of muscle hyperalgesia. This would translate to reduction of bite force and an increase in antagonist muscle activity in the orofacial region. Previously performed experiments in humans³¹⁻³³ have shown this. Hoshino³² has also demonstrated that compared to controls, patients with TMD demonstrate decreased bite force (201 and 223 mV for the masseter muscles respectively in asymptomatic volunteers and 128 and 153 mV for symptomatic patients). Balkhi³³ demonstrated that chewing force was decreased in patients with pain (113 and 102 mV for deliberate right and left side chewing of gum masseter muscles respectively in asymptomatic volunteers and 85 and 83 mV for symptomatic patients). Musgrave et al.³⁴ demonstrated that there was increased jaw muscle activity of antagonists during jaw opening.

Jaw and biting strength has never been investigated in an animal model of TMJ arthritis. Given that TMJ pain correlates with a decrease in bite strength it may be possible to utilize both mechanical testing and histological identification of pain tracks as an endpoint for TMJ disorder therapy. We have preliminary data to show that it is possible to measure the jaw closure strength in both wild type and TNF α transgenic mice. This type of measurement was devised to measure jaw strength in New Zealand white rabbits as part of a continuing investigation on the activation of small diameter unmyelinated and thinly myelinated nociceptive afferent fibers³⁵. The early data for the mouse model indicate that the transgenic animals do, indeed, demonstrate a decrease in biting strength likely due to pain or joint destruction associated with the degeneration of the joint.

Therapeutic strategies to halting the progression of TMJ arthritis

With an animal model that progresses through different stages of tissue destruction and is also amenable to mechanical joint testing, the possibility for therapeutic interventions becomes real. Thus, our goal over the past few years has been to show that anti-TNF α therapy will prevent the downstream pathology in the TMJ mouse model. As this type of therapy has been shown to be effective with histological evaluations, we anticipate a positive result from our trial. In addition, to these "proof-of-principle" studies it should also be possible to determine if anti-TNF α therapy can i) reverse some of the joint destruction, ii) down regulate pain, iii) increase biting strength and iv) be effective if administered intra-articularly, intramuscularly and/or systemically.

Anti-TNF α therapy

As the mouse model develops arthritis due to a sustained level of TNF α , anti-TNF α therapy is likely to be effective.

However, on a broader note anti-TNF α therapy is based on the hypothesis that TNF α is at the apex of the pro-inflammatory cascade that ultimately precipitates joint destruction and synovial inflammation in most arthritic conditions. While this view is shared by a large number of investigators^{23,36} based on experimental data implicating TNF α in the induction of cytokines, adhesion molecules, MMPs, oxygenases and osteoclasts, many remained skeptical about the dominance of TNF α and the potential of anti-TNF α therapy. However, the clinical evidence over the last 4 years supports the view that TNF α is the dominant cytokine in most forms of arthritis and that anti-TNF α therapy dramatically lessens joint pain and swelling and retards bone loss. Multiple placebo controlled, double-blinded, multicenter phase III clinical trials have been performed with two FDA approved anti-TNF α biologics; the soluble p75 TNF α receptor-Fc fusion protein, etanercept (Enbrel®)^{37,38} and the humanized anti-TNF α monoclonal antibody, infliximab (Remicade™)⁴¹. These studies showed that ~70% of patients, who were refractory to two or more DMARDs, (disease modifying arthritis drugs) improved physically, functionally and radiographically. Many of these patients have a sustained remission on these drugs.

Gene therapy for arthritis

Gene therapy was initially devised to correct genetic diseases. As the field advanced, researchers developed additional applications including treatments for cancer, arteriosclerosis, CNS disorders, and AIDS³⁹. Work on gene therapy for arthritis started in the late 1980s⁴⁰, based on the rationale that there was no method to deliver novel therapeutic proteins to the intra-articular site of disease. However, work by Evans et al. has provided evidence that gene therapy might be possible⁴¹. Their primary model has been transducing synovial fibroblasts to express the IL-1R antagonist protein (IRAP)^{42,43}. Using an *ex vivo* gene therapy approach, they were able to suppress antigen-induced arthritis in rabbits, in terms of chondroprotective effects⁴⁴. Based on their experience, they performed a clinical trial to assess the safety, feasibility, and efficacy of IRAP *ex vivo* gene therapy for human RA⁴⁵. While these ground breaking studies are extremely useful as a proof of principle and guidelines for future human gene therapy studies, the results of the trial indicated that *ex vivo* gene therapy for human arthritis is too expensive in time, cost and labor to be a practical therapy.

Over the last four years gene therapy for arthritis has become a very popular area of investigation. However, based on the limitations of *ex vivo* gene therapy, which we have experienced ourselves⁴⁶, many laboratories have been focusing their efforts on *in vivo* gene therapy approaches using a variety of vectors. These include recombinant; naked DNA, retroviruses, adenoviruses, herpes viruses and adeno-associated viruses (rAAV)⁴⁵. We were the first group to evaluate the use of rAAV for arthritis gene therapy back in 1996, based largely on our view that human *in vivo* gene therapy

for arthritis demands: i) a high transduction efficiency that can only be met by viral vectors; ii) a vector that can be delivered at high titer (i.e., 1013/ml); and iii) a vector that does not elicit a dominant/dangerous host immune response. Subsequently we were able to demonstrate that rAAV vectors are very efficient at transducing joint cells *in vivo* (synovocytes, articular chondrocytes and meniscal cells)⁴⁷, as have others⁴⁸. Simultaneously, gene therapy investigators in other fields have also made very important findings regarding the utility of rAAV vectors.

Citing work done on hemophilia as an example of the systemic-secreted gene therapy, breakthrough advances include: i) a direct muscle injection protocol has been developed that routinely delivers between 0.1-80 μ g of gene product per ml of plasma^{49,50}; ii) the demonstration that a single rAAV injection results in stable, long-term (>6 months) gene expression in immune competent mice⁴⁹ and dogs⁵¹, and LacZ expression for more than 1.5 years⁵²; iii) the demonstration that all five AAV serotypes can be used as vectors, which permits multiple gene deliveries⁵⁰; and, iv) human gene therapy trials have shown that there is no evidence of toxicity, germline transmission of vector sequences, or formation of inhibitory antibodies against the target gene product^{56,57}. In studies of gene therapy for arthritis in animal models, several labs have demonstrated the efficacy of rAAV vectors expressing soluble target gene products including: TNFR:Fc⁵⁵, IL-4^{56,57} and IRAP⁵⁸. This has led us to propose that rAAV is the best vector for *in vivo* musculoskeletal gene therapy

Delivery of gene therapeutic agents and Enbrel®

We have been able to demonstrate that rAAV vectors can efficiently transduce multiple cell types in the joint (i.e., synovium, chondrocytes, meniscal cells)⁵⁹, however, efficiency did not approach 100% of the cells. Based on this finding and the remarkable clinical success of systemic anti-TNF α therapy, it is critical to evaluate both intra-articular, intramuscular and systemic protocols for anti-TNF α therapy in TMJ disorders. As we have previously shown, both intra-articular (local) and intramuscular (systemic) administration of the TNF receptor Fc fusion protein (TNFR:Fc gene therapy) leads to similar circulating levels of the protein⁶⁰. Whether one route of administration is more effective than the other for TMJ arthritis remains to be evaluated.

Summary of pertinent points

- 1) The field of TMJ arthritis research has at its disposal a transgenic animal that develops a spontaneous arthritis in the TMJ that in its early stages displays articular chondrocyte cloning, MMP and cytokine expression in the disc cells and chondrocytes and indications of subchondral bone erosion. In its later stages the synovial layers hypertrophy and extensive destruction of carti-

lage, disc and bone can be observed. The staging of these different events is well defined in time.

- 2) All of the histological tools necessary to characterize cell function in articular cartilage, disc, synovium, bone and neurological tissues are available.
- 3) Biomechanical testing on small animal jaw joints is feasible.
- 4) A molecular target with a high chance of success has been identified (i.e., TNF α) that, when blocked, should inhibit the arthritic process.
- 5) Delivery vectors for gene therapy are available and have been shown to work in other mouse models.

Therefore, all that remains is to perform the experiments.

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