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Expression of proinflammatory cytokines in osteoarthritis of the temporomandibular joint

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ABSTRACT

Objective: This study reports the expression of proinflammatory cytokines in temporomandibular joint (TMJ) of patients affected with temporomandibular osteoarthritis (OA).
Design: In twelve OA of the TMJ (OA–TMJ) affected patients and in six healthy volunteer subjects studied as control, the expression of IL1β (interleukin-1β), IL2, IL4, IL5, IL6, IL10, IL12p35, IL12p40, IL17, IFNγ (interferon-gamma), TNFα (tumor necrosis factor-alpha), and TNFβ mRNAs was evaluated. Using quantitative real-time RT-PCR technique, the cytokine levels, reported as Ct (cycle threshold), ΔCt (Ct cytokine − Ct 18S rRNA) and RQ (relative quantification), in patient and control groups were compared.
Results: Expression of IL1β, IL2, IL12p35, IL12p40, IL17, TNFα, TNFβ, and IFNγ mRNAs was significantly higher in patients as compared with controls. In particular, IL12 was the predominant cytokine expressed in patients (IL12p35 RQ = 30.2 and IL12p40 RQ = 29.0). Conversely, IL10 mRNA levels were higher in controls (RQ = 1.8).
Conclusions: These data suggest that not only IL1β, IFNγ, and TNFα but also IL10, IL12, and IL17 are involved in the OA–TMJ pathogenesis. Furthermore, an inflammatory response characterised by the predominant expression of IL12 mRNA and down-regulated expression of IL10 mRNA is associated with the degenerative changes observed in OA–TMJ.

1. Introduction

Temporomandibular disorders (TMD) are a group of conditions affecting the masticatory muscles, the temporomandibular joints (TMJ), and their associated anatomic structures, with a different aetiology and clinical presentation. Osteoarthritis (OA) is a degenerative disease affecting the TMJ. It is the most common TMD and shows a higher prevalence in women and older age groups.[1–3] OA of the TMJ (OA–TMJ) is characterised by variable degrees of inflammation and abrasion of the articular cartilage and bone.[4,5] Various inflammatory mediators, bone-destruction associated cytokines, and metallo-proteinases (MMPs) have been considered as possible markers of active OA–TMJ, including interleukin (IL) 1β, IL6, interferon-gamma (IFNγ), tumor necrosis factor-alpha (TNFα), prostaglandin E_2 (PGE_2), receptor activator of NF-κB ligand (RANKL), osteoprotegerin (OPG), MMP-2, MMP-9, aggrecanase-1, and aggrecanase-2.[6–11]

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Although inflammatory-bone and/or cartilage destruction diseases, such as rheumatoid arthritis (RA) and chronic periodontitis (CP), have been traditionally categorized as Th1-type diseases, Th1 cells do not have osteoclastogenic characteristics activity, indicating that the osteoclastogenic cells might belong to an as-yet unknown Th subset.[12–15] Recent data have indicated that an IL17 producing Th cell subset is responsible of the bone damage observed in autoimmune arthritis [15,16] and it has been reported that IL17, rather than IL12 or IFNγ, is critical in the onset phase of the disease.[17,18] Moreover, it has been reported that IL17 is detectable in the synovial fluid from RA patients and enhances bone destruction after induction of RANKL expression.[19] In OA–TMJ, Th1 cytokines are not always highly expressed in the lesion, thus, not only IL1β, IL12 or IFNγ but also IL17 producing cells could be related to its pathogenesis.

The cytokine balance, their receptors, and receptor antagonists are determinants not only for initiation and progression, but also for the clinical expression of these diseases. Recent advances in the biochemical analysis of synovial fluid from affected patients have provided new insights into the pathophysiology of the TMJ disorders; however, the molecular basis of OA etiopathogenesis remains still unclear. Association of IL12 with TMJ disorders has been scarcely studied and IL17 expression in OA–TMJ has not been previously described. This study aimed to determine the cytokine expressions from cells sampled from the TMJ of OA affected patients.

2. Materials and methods

Samples of TMJ synovial fluid were collected from twelve patients (four males and eight females, aged 41.4 ± 2.4) diagnosed of OA, based on their clinical symptomatology and ratified by magnetic resonance and radiographic images. As control, six healthy volunteers (two males and four females, aged 39.0 ± 2.2) without a history of TMD and lack of TMD-related symptoms were studied. All subjects signed an informed consent approved by the Institutional Review Board of the University Complutense de Madrid. Inclusion criteria for patient enrolment were the presence of TMJ soreness, impaired joint mobility, and presence of images characteristic of degenerative changes of the condyle, such as flattening and erosion. Exclusion criteria were diagnosis of chronic systemic inflammatory diseases or previous treatment of TMJ disorders. Evaluation and data registration were performed by two independent oral surgeons. Patients were given no medication for at least 2 weeks before the synovial fluid sampling was performed.

2.1. Sample collection

After extracapsular local anesthesia, the synovial fluid was collected by puncture with a 21-gauge needle inserted inferolaterally into the superior joint space. Two milliliters of normal saline solution was injected and, after 1 min mixing by repeated opening and closing of the patient's mouth, 2 mL of synovial fluid and saline solution mixture was aspirated. Samples were devoid of blood contamination. The cells present in these samples were recovered by centrifugation at $300 \times g$ for 10 min and subsequently resuspended in 5 mL of sterile culture medium RPMI 1640, supplemented with 50 UI/mL penicillin, 50 μg/mL streptomycin, 200 mM L-glutamine (Sigma Chemical Co, St. Louis, MO, USA) and 10% foetal bovine serum (Gibco Invitrogen, Grand Island, NY, USA). Cell counting was performed on a Neubauer chamber using a phase contrast microscope and cell viability was calculated by Trypan blue dye exclusion. In the analysed samples, cell viability was always equal to or greater than 90%. The cell suspensions obtained from the synovial fluid were then used to prepare the RNAs used for this study.

2.2. Cytoplasmic RNA isolation

Cytoplasmic RNA was isolated as previously described.[20] For each subject, 10^6 cells were washed twice in PBS for 10 min at $300 \times g$ at room temperature, transferred to a microcentrifuge tube in 1 mL of PBS, centrifuged for 10 s at $14\,000 \times g$ at room temperature and lysed in 400 μL of ice-cold lysis buffer containing 0.5% Nonident P-40, 50 mM Tris HCl pH 8, 100 mM NaCl and 5 mM $MgCl_2$, supplemented with 10 mM VRC 40 (Gibco). Complete cell lysis was obtained by pippeting up–down 10 times, and the nuclei were removed by centrifugation for 10 s at $14\,000 \times g$. Four hundred microliters of the supernatant were digested with 25 μg/mL proteinase K (Roche Ltd., Basel, Switzerland), in the presence of 1% SDS and 15 mM EDTA (Fluka) for 30 min at 37 °C. Subsequently, RNA was extracted with an equal volume (400 μL) of chloropan (phenol:chloroform:isoamylalcohol 25:24:1) and centrifuged for 10 min at $14\,000 \times g$ at room temperature (Fluka). The RNA present in the aqueous solution was precipitated for 1 h at −20 °C with 0.3 M Na-acetate, 2.5 volumes 100% EtOH and 1 μL glycogen 20 μg/μL (Roche), recovered by centrifugation for 30 min at $14\,000 \times g$, and resuspended in 20 μL H_2O RNase-free. RNA quality was determined in a bioanalyser (Agilent 2100B, Agilent Tech., Palo Alto, CA, USA). Cytoplasmic RNA was quantified using a spectrophotometer (Nanodrop ND-1000, Nanodrop Tech., Wilmington, ND, USA) and stored at −80 °C at a final concentration of 1 μg/μL.

2.3. First-strand cDNA synthesis

Reverse Transcription (RT) was performed using a Transcriptor First Strand cDNA synthesis kit (Roche) following the manufacturer's recommendations. Briefly, 20 μL reaction containing: 4 μL 5× RT buffer, 0.5 μL RNase inhibitor (20 U), 2 μL deoxynucleotide mix (1 mM each), 2 μL random hexamer primer (60 μM), 0.5 μL reverse transcriptase (10 U), 10 μL RNA-grade H_2O, and 1 μL RNA sample (1 μg) were retrotranscribed under the following conditions: 10 min at 25 °C and 1 h at 50 °C (Primus 96 plus, MWG Biotech AG, Ebersberg, Germany). The reverse transcriptase activity was subsequently inactivated by incubating the sample at 85 °C for 5 min.

2.4. Quantitative real-time PCR

The mRNA expression levels of the following cytokines: IL1β, IL2, IL4, IL5, IL6, IL10, IL12p35, IL12p40, IL17, IFNγ, TNFα, and TNFβ were quantified using real-time quantitative PCR. Fifty