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ORIGINAL ARTICLE

Inflammatory cell and cytokine patterns in patients with chronic polyarthritis and temporomandibular joint involvement

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Abstract

Objective. To investigate the occurrence of selected markers for inflammatory cells and cytokines in patients with chronic polyarthritis (CPA) and temporomandibular joint (TMJ) involvement. ***Material and Methods***. Eleven patients (11 joints) with CPA and TMJ disorder were included in the study. Synovial specimens were obtained during TMJ open surgery and these were subjected to immunohistochemistry on frozen sections post-fixed with paraformaldehyde and with the cell membranes permeabilized by saponin. In all patients, the cytokines IL-1α, IL-1β, IL-1ra, TNFα, IFNγ, IL2, and TGFβ were investigated using specific antibodies. The occurrence of macrophages and T-lymphocytes was investigated using immunohistochemistry with monoclonal antibodies against antigens CD68 and CD45RO, respectively. In addition, PCNA was used as a marker for cell proliferation. ***Results***. Staining of IL-1α, IL-1, and TGF was seen in all 11 specimens, IFN? in 1, TNFα in 4, and IL-2 in none. CD45RO-positive T cells were detected in 7 specimens, CD68-positive macrophages in 6, and cell proliferation seen with PCNA was noted in 8. ***Conclusions***. The predominant cytokines of TMJ CPA were IL-1α, IL-1β, and TGFβ, and there appeared to be no differences between the subgroups (rheumatoid arthritis, psoriatic arthritis, and ankylosing spondylitis) involved. Moreover, the cytokine pattern of TMJ CPA patients seemed to differ from patients with osteoarthritis, as shown in our previous study. The main difference was the absence of IFNγ and TNFα in TMJ CPA patients and a stronger TGFβ and IL-1α expression.

Key Words: *Cytokines, inflammation, monoclonal antibodies, synovial biopsies, temporomandibular joint*

Introduction

Chronic polyarthritis (CPA) may present in the temporomandibular joint (TMJ). The most common form is rheumatoid arthritis (RA), followed by psoriatic arthritis (PA) and ankylosing spondylitis (AS). Previous studies have implied the influence of genetic factors [1] and inflammatory mediators such as cytokines and their receptors are most likely involved in the pathogenesis [2]. The involvement of the TMJ may result in clinical findings such as tenderness to palpation, crepitation, pain on mandibular movements, anterior open bite, and, radiographically, erosion, sclerosis, and flattening of the articular surfaces [3–6]. Inflammatory cell markers (CD45RO, CD68, and a marker for cell proliferation, PCNA) have proved useful in characterizing the pathological process in the TMJ in patients with painful clicking and osteoarthritis, and high levels of these markers have shown a correlation with arthroscopic signs of inflammation [4].

Cytokines are involved in the regulation of the inflammatory response where they may act as mediators, being either inhibitory or synergic [7]. Cytokines released during inflammation may induce protease production, resulting in cartilage degradation [8,9]. The pro-inflammatory cytokines, interleukin-1α,β (IL-1α,β) and tumor necrosis factor alpha (TNFα), produced mainly by monocytes and macrophages, cause inflammatory diseases [2,10]. IL-1α, β and TNFα may stimulate collagenase and

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Table I. Preoperative clinical variables in 11 patients with chronic arthritis. RA = rheumatoid arthritis, AS = ankylosing spondylitis, PA = psoriatic arthritis, MFIQ = mandibular function impairment questionnaire (normal <8), VAS = visual analog scale (normal <2), MIO = maximum interincisal opening in millimeter (normal >40), MP = maximum protrusion in millimiter (normal >6), JT = tenderness on palpation of the lateral joint, MT = tenderness on palpation of the jaw muscle (>one muscle on the affected side). Y = Yes, N = No

Patients	RA 1	RA 2	RA 3	AS 4	RA 5	PA 6	RA 7	AS 8	RA 9	PA 10	PA 11
MFIQ	21	18	20	13	22	13	21	21	11	22	14
VAS	4	6	8	7	8	7	8	7	5	7	4
MIO	25	23	23	30	25	38	20	20	24	16	30
MP	8	5	6	7	6	9	5	7	2	0	7
JT	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
MT	Y	Y	Y	Y	N	N	Y	Y	Y	Y	Y

prostaglandin E production resulting in cartilage and bone destruction [11]. Interleukin-1 receptor antagonist (IL-1ra) is also present in the joints of patients with RA, and acts as a natural inhibitor of IL-1 [12,13]. Interferon gamma (IFNγ) is a potent activator of macrophages, synoviocytes, and chondrocytes and has been reported to interact with IL-1 [14]. IL-2 is produced mainly by T-helper cells and may induce the outgrowth of synovial fibroblasts [15]. Transforming growth factor (TGFβ) is produced by a variety of cells, including macrophages, lymphocytes, and synovial fibroblasts [16]. Its primary function is to stimulate matrix synthesis and inhibit matrix degradation.

In our previous studies, we found patients with painful clicking and osteoarthritis to have clearly different matrix glycosaminoglycan [17] and inflammatory cell and cytokine patterns [18]. In this study, our aim was to investigate the occurrence of the above-described markers for inflammatory cells and cytokines in patients with CPA and TMJ involvement.

Material and methods

The material comprised consecutive patients referred to our department and who had then been selected for TMJ discectomy. Our criteria for discectomy were unsuccessful non-surgical treatment (at least 3 months) and painful impaired TMJ mobility (VAS <2 and MFIQ <8; see below). The surgical technique for discectomy was the same as that used in our previous study [5].

The inclusion criteria of the study were: a diagnosis of CPA verified by a specialist in rheumatology according to accepted criteria [19,20], radiographic signs of TMJ CPA (erosion and sclerosis) on the preoperative tomograms, and a painful impaired mobility of the TMJ. The exclusion criteria were other TMJ diseases (internal derangements, osteoarthritis, and other polyarthritis than RA, PA, and PS), major jaw trauma, and dento-facial deformity. During discectomy, synovial specimens were taken from the posterior disk attachment in 11 patients (11 TMJ). Each biopsy was divided into two specimens, one for the cytokine study, the other for the cell study. The mean age was 49 years (range 28–68). There were 9 females and 2 males with a mean duration of TMJ symptoms of 4 years (range 1.5 to 12). The clinical examinations were done by one of the authors (R.K.) immediately before surgery. All patients were asked to complete the mandibular function impairment questionnaire (MFIQ) [22]. A 10-cm visual analog scale (VAS) was used to assess pain with the end-points marked score 0 = “no pain” and score 10 = “worst pain ever experienced”. The patients were asked to estimate their pain intensity during repeated jaw movements (maximum opening and maximum protrusion). Maximum interincisal opening and maximum protrusion were measured with a ruler to the nearest millimeter. The preoperative clinical signs and symptoms and medication of the patients are presented in Tables I and II. The radiographic examination comprised computed tomograms in the intercuspal position with the tomographic plane perpendicular to the long axis of the condyle.

Immunohistochemistry for cytokines

The staining procedure has been described in detail previously [23]. Briefly, the biopsies were snap-frozen and kept in a freezer at −70°C pending staining. Cryostat sections, 7-μm thick, were mounted on glass slides and fixed in phosphate buffered 2% paraformaldehyde (PFA). The slides were then washed in Earl’s balanced salt solution (EBSS) containing 0.1% saponin. The endogenous peroxidase was blocked with 1% hydrogen peroxide and 2% sodium nitride dissolved in EBSS saponin. The slides were then washed in EBSS saponin and incubated in a humid chamber overnight with the selected panel of cytokine-specific, affinity-purified antibodies at a concentration of 2–5 μg/ml in EBSS saponin (Table III). Control staining with irrelevant subclass-specific antibodies was performed in parallel. After incubation, the slides were washed in EBSS saponin and incubated with 1% normal goat serum in EBSS saponin. Secondary biotin-labeled antibodies, absorbed against human Ig, were used

Table II. Medication of the patients in the study

	Patients	Duration of symptom (year)	Steroid injection in TMJ	Medication		
				NSAID	DMARDS	Steroids
F	1	2	Yes 2 months before surgery	Yes	No	Yes
F	2	2	No	Yes	Yes (Imurel)	Yes
M	3	1	No	Yes	No	No
F	4	3	No	Yes	No	No
F	5	2	No	Yes	No	No
M	6	1	No	No	No	No
F	7	1	No	Yes	Yes (Sandimmun Methrotrexate)	No
F	8	5	No	Yes	No	No
F	9	5	No	Yes	Yes (Methotrexate)	No
F	10	13	No	Yes	No	No
M	11	20	No	No	Yes (Methotroxate, Enbrel)	Yes

DMARDS = Disease modifying anti-rheumatic drugs. Imurel (Azathioprine) – immunosuppressive; Sandimmun (Cyclosporine) – immunosuppressive; Methotrexate – immunosuppressive; Enbrel (Etanerecept)-TNFα inhibitor.

as a first step in the detection procedure. The slides were washed in EBSS saponin and then incubated with avidin. After another washing in EBSS saponin, the reaction product was visualized by diaminobenzidine. After 10 min the slides were washed in EBSS counterstained with Mayer's hematoxylin and mounted in a glycerine buffer. The cytokine staining was assessed as follows: no cell staining was recorded as 0, 1–5 stained cells per section as 1, 6–10 stained cells as 2, and more than 10 stained cells as 3. All microscopic examinations were performed by two of the authors in consensus (A.U. and R.K.).

Immunohistochemistry for inflammation markers

This procedure has also been described in a previous publication [4]. In essence, the specimens were fixed in 4% phosphate-buffered formalin, embedded in paraffin, and cut into 3-μm-thick sections. The sections were incubated with antibodies against the following human epitopes: DAKO, monoclonal mouse anti-human macrophage (CD68, clone KP1), monoclonal mouse anti-human T cell (CD45RO, clone UCHL), monoclonal mouse anti-proliferating cell nuclear antigen (PCNA clone PC10). The bound antibodies were visualized using the avidin-biotin complex (ABC) method and counterstained with hematoxylin. Subclass-specific antibodies were used for negative control staining. One set of sections was stained with hematoxylin-eosin according to routine protocol. Monoclonal antibody staining was assessed as follows: no stained cells were recorded as 0, focal occurrence of stained cells as 1, and several foci/large numbers of stained cells as 2. Immunohistochemical staining of the inflammatory cells was recorded in the lining layer (LL) and in the deeper parts (D) of the specimens. All microscopic evaluations were performed by two of the authors together (A.U. and R.K).

Results

Arthrotic changes were observed during surgery on the articular surfaces and the disk in all cases. These findings correlated well with the preoperative radiographic findings. Fibrotic bands were also frequently observed. It was not possible to determine disk position. The disk was too degenerated. Staining of IL-1α (Figure 1A), IL-1 (Figure 1B), and TGF (Figure 1C) was seen in all specimens, TNFα in 4, and IFN? in only 1. IL-2 and IL-1ra were not stained in any specimen. The scores of the cytokines are given in Table IV.

As regards the inflammatory cell response, CD45RO-positive T cells (Figure 2A) were detected in 7 specimens, CD68-positive macrophages (Figure 2B) in 6 specimens, and cell proliferation seen with PCNA (Figure 2C) in 8. The scores are given in Table V.

Table III. Cytokine-specific antibodies used for tissue staining

Cytokine	Antibody	Isotype	Producer
IL-1α	1277-89-7,1277-82-29,1279-143-4	Mouse IgG1	Immunokontakt Bioggo, Switzerland
IL-1β	2-D-8+1437	Mouse IgG1	Immunokontakt Bioggo, Switzerland
IL-1ra	1384-92-17-19	Mouse IgG1	Biochemicals, Switzerland
TNFα	Mab1+Mab11	Rat IgG	Pharmingen, USA
IFNγ	DIK1,7B6	Mouse IgG1	Pharmingen, USA
IL-2	MQ1-17H12	Rat IgG2a	J. Abrams, DNAX, Palo Alto, Calif., USA
TGFβ	K96	Polyclonal. rabbit. IgG	K. Miyazono, Ludwig Institute, Sweden

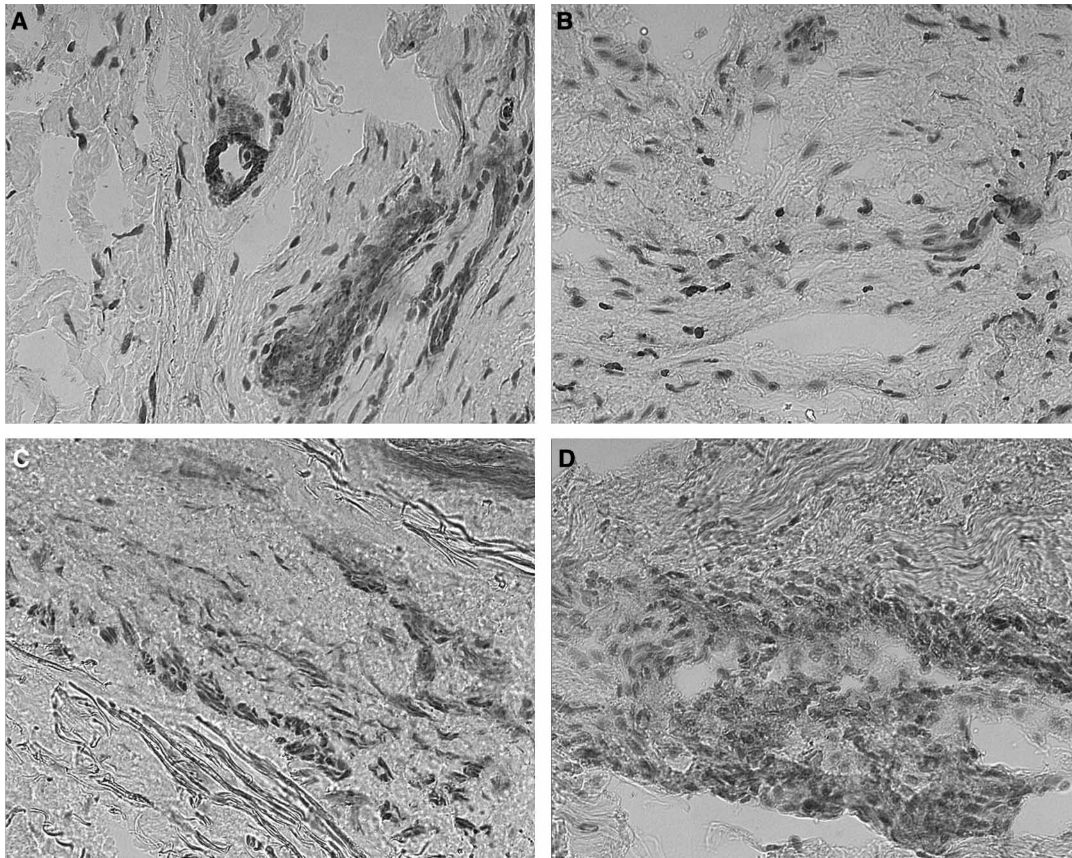

Figure 1. A. CA specimen: Immunohistochemical staining showing cells containing IL-1α. B. Immunohistochemical staining showing cells containing IL-1. C. Immunohistochemical staining showing cells containing TGF. D. Immunohistochemical staining showing cells containing TNFα. Original magnification ×10.

Discussion

Our method for detecting the cytokines made it possible to assess the intracellular content of the cytokines, which means that the results ought to be more relevant to the process in the tissue than those obtained from methods such as synovial washout [22]. Synovial washout cannot measure the cytokine activity at the site where inflammation is most prominent (the posterior disk attachment). When it works (sometimes it is not possible to get a sample) it rather reflects the sum of all cytokines released locally and from serum. We also used markers for inflammatory cells to find the cells in the area of cytokine activity. We did not use control material in this study, although this would have been ideal. For ethical reasons, surgical specimens cannot be obtained from normal controls. However, we were able to compare our findings with those of a previous study on TMJ osteoarthritis and painful clicking. This study used the same methods as the present one and investigated the same cytokines and inflammatory cells.

Another limitation with the study is the small material. It took us more than 5 years to gather the 11 patients for this study, as most patients with TMJ CPA seem to improve after only medication and non-surgical treatment.

The main cytokines were IL-1α, IL-1, and TGF, and we found no differences when comparing patients with RA, PA, and AS. However, it has to be admitted that the patients under study, and especially those with AS and PA, were few. IL-1α was almost always confined to endothelial cells, as observed in our previous study comprising patients with osteoarthritis [16]. It thus seems that the expression of IL-1α is not specific for TMJ CPA. Compared to patients with TMJ osteoarthritis, the CPA patients of this study showed a stronger expression of IL-1α [18]. IL-1α may play a role in the inflammatory process by regulating the angiogenesis and permeability of vessels. A more

Table IV. Occurrence of cytokines and their scores in patients with TMJ CA, where 0 = no stained cells, 1 = 1–5 stained cells per section, 2 = 6–10 stained cells, and 3 = more than 10 stained cells

Patients	1	2	3	4	5	6	7	8	9	10	11
IL-1α	3	2	2	2	2	3	2	2	3	3	2
IL-1	2	3	2	2	1	2	2	1	1	2	1
IL-1ra	0	0	0	0	0	0	0	0	0	0	0
IFNγ	0	0	0	0	0	1	0	0	0	0	0
TNFα	1	1	1	0	0	1	0	0	0	0	0
IL2	0	0	0	0	0	0	0	0	0	0	0
TGF	2	2	2	2	2	2	3	3	3	3	2

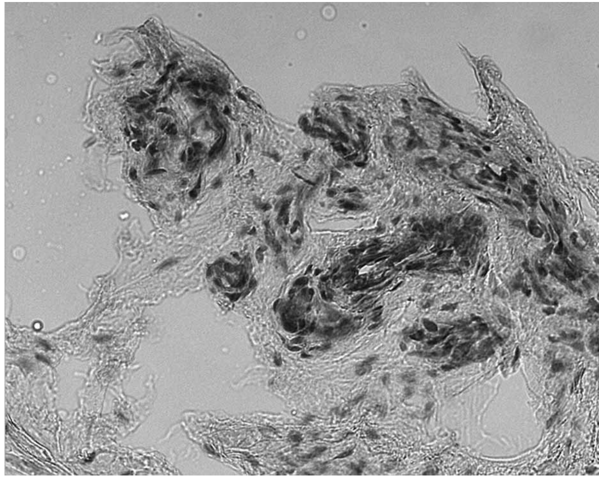

Figure 2. CA specimen: Micrograph showing grade 2 staining with antibodies against PCNA (marker for cell proliferation).

pronounced vascularization was also typical for patients with TMJ RA compared with patients with TMJ osteoarthritis and internal derangement in a previous arthroscopic study [23]. Our results therefore indicate that IL-1α may play a stronger role in the disease process in TMJ CPA. The expression of IL-1β did not differ much from what was previously found for TMJ osteoarthritis [18]. One would have expected a stronger expression in the CPA patients, as indicated in a previous study [22]. The release of IL-1 has also been attributed to macrophages in a previous study [24]. However, the expression of macrophages was low and the IL-1 activity may therefore be attributed to other cells, possibly fibroblasts.

In our previous study on patients with TMJ osteoarthritis, IFN? and TNFα were also frequently stained [18]. TNFα is regarded as a potent inducer of synovitis and promotes the production of proteinases resulting in degradation of cartilage [8]. Cartilage destruction was a frequent finding during surgery and the infrequent staining of TNFα is therefore puzzling. One explanation may be that some patients were treated with disease-modifying drugs which might have suppressed the cell activity.

Table V. Results of the immunohistochemical staining of T cells, macrophages, and proliferating cells showing scores where 0 = no stained cells, 1 = mild: focal occurrence of stained cells, and 2 = moderate: several foci/large numbers of stained cells. LL = lining layer, i.e., surface/synovial membrane and the immediate underlying tissue. D = deeper layer in the tissue

Patients		1	2	3	4	5	6	7	8	9	10	11
CD45R0	LL	0	0	1	0	1	0	0	0	0	0	0
	D	0	1	1	0	1	0	1	1	0	1	1
CD68	LL	0	1	1	0	1	0	0	0	0	1	1
	D	0	1	1	0	1	0	0	1	0	1	2
PCNA	LL	1	1	1	0	0	1	1	1	0	0	2
	D	1	2	1	0	1	1	1	2	0	0	2

The cell activity was also low in this study (Table IV). However, also patients who only had NSAID medication showed the same features (Table II). Schumacher et al. [25] found that NSAID treatment for 2 weeks increased the levels of TNFα in joint synovial fluid. Our results contradict their finding. One explanation could be that the medication in our patients had continued for a long time.

TGFβ was also frequently stained in the CPA patients. Although TGFβ may induce inflammation early in the disease process its main function is to stimulate matrix sythesis. Therefore, in this patient material, where symptoms had persisted for several years, the high TGFβ activity should be interpreted rather as a sign of increased repair activity.

In conclusion, to our knowledge this study is the first to investigate the cytokine and inflammatory cell patterns in synovial biopsies from patients with CPA and TNJ involvement. The predominant cytokines of TMJ CPA were IL-1α, IL-1β, and TGFβ and there appeared to be no differences between the patient groups involved. Moreover, the cytokine pattern of TMJ CPA patients seemed to differ from patients with osteoarthritis, as shown in our previous study [18]. The main difference seems to be related to the absence of IFNγ and TNFα in TMJ CPA patients and a stronger TGFβ and IL-1α expression.

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