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TAURINE CHLORAMINE INHIBITION OF CELL PROLIFERATION AND CYTOKINE PRODUCTION BY RHEUMATOID ARTHRITIS FIBROBLAST-LIKE SYNOVIOCYTES

EWA KONTNY, AGNIESZKA GRABOWSKA, JACEK KOWALCZEWSKI, MARIOLA KUROWSKA, IWONA JANICKA, JANUSZ MARCINKIEWICZ, and WŁODZIMIERZ MAŚLIŃSKI

Objective. **To examine whether taurine (Tau) or its physiologic chlorinated derivative, taurine chloramine (Tau-Cl), affects proliferation of, and proinflammatory cytokine (interleukin-6 [IL-6] and IL-8) production by, fibroblast-like synoviocytes (FLS) isolated from rheumatoid arthritis (RA) patients.**

Methods. **FLS, isolated from the synovial tissue of 19 RA patients and cultured in vitro for 3–6 passages, were stimulated with the recombinant human cytokines IL-1β (1 ng/ml), tumor necrosis factor α (TNFα; 10 ng/ml), or IL-17 (10 ng/ml) in the presence of either Tau or Tau-Cl, which were added at concentrations of 50–500 μM. Tau and Tau-Cl were added simultaneously with, 2 hours before, or 24 hours after the stimuli. The concentrations of IL-6 and IL-8 were determined in culture supernatants using specific enzyme-linked immunosorbent assays. Proliferation of FLS was estimated on the basis of ^{3}H-thymidine incorporation into the cells, which were cultured for 72 hours in the presence of recombinant human basic fibroblast growth factor (bFGF) (1 ng/ml) and Tau or Tau-Cl, which were added simultaneously at the beginning of the culture.**

Results. **Cultured in vitro, RA FLS spontaneously secreted low levels of IL-6 and IL-8, but when RA FLS were stimulated with IL-1β, TNFα, or IL-17, significantly higher amounts of IL-6 and IL-8 were produced. Tau-Cl, but not Tau, inhibited cytokine-triggered synthesis of IL-6 (50% inhibitory concentration [IC_{50}] $\sim$225 μM) and IL-8 (IC_{50} $\sim$450 μM) when added simultaneously with the stimuli. However, IL-17–induced production of IL-8 was not affected by Tau-Cl. In the cells prestimulated with IL-1β for 24 hours, Tau-Cl still inhibited synthesis of IL-6, but did not affect IL-8 production. Moreover, Tau-Cl inhibited spontaneous and bFGF-triggered proliferation of FLS in a dose-dependent manner. Neither Tau nor Tau-Cl affected cell viability.**

Conclusion. **The results of these studies demonstrate that Tau-Cl inhibits production of proinflammatory cytokines by RA FLS, as well as proliferation of these cells. Thus, Tau-Cl may act as a physiologic modulator of FLS functions related to their pathogenic role in RA.**

Rheumatoid arthritis (RA) is characterized by chronic inflammation of the synovium with hyperplasia in the synovial lining cells (1). Accumulating evidence supports the opinion that fibroblast-like synoviocytes (FLS) from RA patients are active participants in synovitis. These cells predominate in pannus and are engaged both in the destructive process (by secreting enzymes that destroy nearby tissues) and in the cytokine network (for review, see ref. 2). It is well known that in the joint milieu created by the presence of various cytokines (e.g., tumor necrosis factor α [TNFα], interleukin-1β [IL-1β], IL-17) (2–4) and growth factors (e.g., platelet-derived growth factor [PDGF], fibroblast growth factor [FGF]) (5,6), RA FLS proliferate and secrete IL-6 and IL-8. Both IL-6 and IL-8 are thought to participate in the pathogenesis of RA.

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Ewa Kontny, PhD, Jacek Kowalczewski, MD, Mariola Kurowska, MSc, Iwona Janicka, MSc, Włodzimierz Maśliński, PhD: Institute of Rheumatology, Warsaw, Poland; Agnieszka Grabowska, PhD, Janusz Marcinkiewicz, PhD, MD: Jagiellonian University Medical College, Cracow, Poland.

Address reprint requests to Ewa Kontny, PhD, Department of Pathophysiology and Immunology, Institute of Rheumatology, Spartanska 1, 02-637 Warsaw, Poland.

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IL-6 supports proliferation of synovial cells (7,8), regulates proliferation and differentiation of osteoclasts, thus supporting bone resorption (9), and induces acute-phase proteins (10). In contrast, IL-8 is postulated to participate in RA synovitis due to its chemotactic activities, its ability to activate neutrophils and macrophages, and its ability to induce formation of new blood vessels in synovial membrane (10,11). Although the cytokine network may account for some aspects of the aberrant function of RA FLS, these cells also exhibit some characteristics reminiscent of transformed cells: anchorage-independent growth, oncogene expression (c-myc and c-fos, mutation in p53 gene) (2,12), or invasive behavior on normal human cartilage engrafted into SCID mice (13). Many therapeutic approaches for immune intervention in RA have been proposed, including anticytokine strategies (directed toward TNFα, IL-1β, IL-6), application of antiinflammatory cytokines such as IL-4 and IL-10 (14), or approaches that interfere with the functions of FLS (2,12).

In the present study, we focused on taurine chloramine (Tau-Cl), a physiologic factor generated in activated neutrophils from taurine (Tau) by the myeloperoxidase-H_2O_2-chloride system (15). Although the metabolism of Tau/Tau-Cl in RA patients has not been evaluated in detail, and data concerning concentrations of Tau/Tau-Cl in the synovial fluid of RA patients are not available, some observations have suggested a disturbance in the metabolism of Tau in these patients. These observations include the following: 1) neutrophils within RA synovial fluid are primed and activated, and secrete granule enzymes, including myeloperoxidase (16); 2) elevated levels of Tau have been detected in plasma (17); and 3) hypertaurinuria has been noted in RA patients (18).

Tau-Cl (for review, see ref. 15) is produced during a respiratory burst in activated neutrophils and, to a lesser extent, in monocytes, in a reaction where the most abundant free amino acid, Tau-2-aminoethanesulfonic acid, serves as a scavenger of the myeloperoxidase-produced oxidant, hypochlorous acid (HOCl). It is proposed that Tau, present in the cytosol of leukocytes, protects these cells from the attack by chlorinated oxidants that escape from phagolysosomes, while extracellular Tau gives such protection to the blood cells and tissues. Tau-Cl is a less reactive and more stable oxidant than HOCl. The recently discovered biologic activities of Tau-Cl support the opinion that it represents more than an end product of $HOCl/OCl^-$ detoxification. For example, Tau-Cl inhibits in vitro the production of various inflammatory mediators and the generation of tissue-damaging factors (nitric oxide, prostaglandins, TNFα, IL-6) in both neutrophils and macrophages (19–22).

Therefore, the aim of this study was to determine whether Tau-Cl and its precursor, Tau, affect certain functions of RA FLS in vitro that are related to the pathogenic role of these cells in RA.

Table 1. Clinical characteristics of the rheumatoid arthritis patients*

Characteristic	Value
No. of patients	19
Age (range), years	55.4 ± 3.1 (26–78)
Sex, no. female/male	17/2
Disease duration (range), years	14.7 ± 2 (2–30)
Radiologic grade, no. of patients†	
II	4
IV	5
V	10
IgM rheumatoid factor positivity, no. (%) of patients‡	10 (53)
ESR (range), mm/hour	34.6 ± 3.1 (10–65)
WBC (range), mm^3	8,547 ± 714 (4,500–16,900)

* Except where otherwise indicated, values are the mean ± SEM. ESR = erythrocyte sedimentation rate; WBC = white blood cells.
† As defined by Larsen et al (24).
‡ By Waaler-Rose hemagglutination test.

PATIENTS AND METHODS

Patients, synovial samples, and synoviocyte cultures. All patients included in this study fulfilled the American College of Rheumatology (formerly, the American Rheumatism Association) criteria for the diagnosis of RA (23). The clinical profiles of the patients are shown in Table 1. Samples of rheumatoid synovium were obtained from 19 patients who were undergoing knee synovectomy or joint replacement surgery as a normal part of clinical care. Tissue was processed within 2 hours after removal from the patient.

After discarding fat and fibrous tissues, the synovium was cut into small pieces, washed with RPMI 1640 medium, and digested with 0.25% trypsin (Sigma, St. Louis, MO) in sterile RPMI 1640 medium for 15–30 minutes at 37°C. The tissue was then filtered using a fine sterile gauze, washed, and resuspended in complete medium made of RPMI 1640 medium supplemented with 2 m*M* L-glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin, 50 μg/ml gentamycin, 20 m*M* HEPES buffer, and 10% fetal calf serum (FCS). The cells were counted, seeded into culture flasks (2.5×10^4 cells/cm^2), and cultured overnight in a humidified, 5% CO_2 atmosphere at 37°C. The nonadherent cells were then washed out. Adherent cells were cultured in complete medium and, at confluence, were passaged into fresh culture flasks after trypsin/EDTA (Sigma) treatment. Synoviocytes were used for experiments between passages 3 and 6. At that time, all cells showed fibroblast-like morphology and did not secrete cytokines originated from macrophage-like synoviocytes (TNFα, IL-1β) (data not shown).

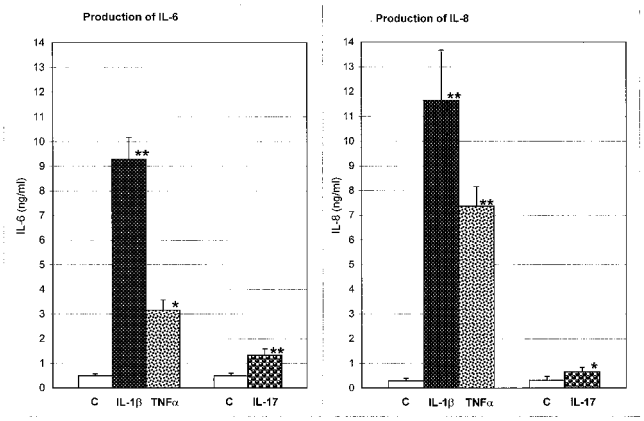


Figure 1. Production of interleukin-6 (IL-6) and IL-8 by fibroblast-like synoviocytes (FLS) of rheumatoid arthritis (RA) patients. Cells (2×10^4/ml) were cultured for 24 hours in the absence (control [C]) or presence of recombinant human IL-1β (1 ng/ml), tumor necrosis factor α (TNFα; 10 ng/ml), or IL-17 (10 ng/ml). Concentrations of IL-6 and IL-8 were determined in culture supernatants by enzyme-linked immunosorbent assay. Results represent cytokine production by FLS isolated from the synovial tissues of 19 RA patients (IL-1β– and TNFα-triggered responses) or 10 RA patients (IL-17–induced response). Bars show the mean and SEM. * = P = 0.01–0.001; ** = P = 0.001–0.0001 versus control.

Synthesis of Tau-Cl. Taurine monochloramine (Tau-Cl) was prepared according to the method described previously (20). Briefly, 5 ml of 20 m*M* NaOCl (Aldrich, Steinheim, Germany) solution in 0.2*M* phosphate buffer (pH 7.4–7.5) was added dropwise to 5 ml of 24 m*M* Tau (Sigma) with vigorous stirring. Each preparation of Tau-Cl was monitored by ultraviolet absorption spectra (200–400 nm) to assure the authenticity of Tau-Cl (252 nm) and the absence of dichloramine, NH_2Cl, and unreacted $HOCl/OCl^-$. The concentration of synthesized monochloramine was determined by the molar extinction coefficient 415 M^{-1} cm^{-1} at A_{252}. Stock solutions of Tau and Tau-Cl (10 m*M*) were kept at 4°C for a maximum period of 4 days before use.

Analysis of cytokine production in FLS by enzyme-linked immunosorbent assay (ELISA). For the assay of cytokine production, 24-well, flat-bottom culture plates were seeded with 2×10^4 cells in culture medium. After 2–3 days of culture, fresh complete medium supplemented with recombinant human IL-1β (1 ng/ml) or TNFα (10 ng/ml) or IL-17 (10 ng/ml) was added for another 24 hours. IL-1β and TNFα were from R&D Systems (Abingdon, UK), while IL-17 was a gift from Immunex (Seattle, WA). The cells were treated with Tau/Tau-Cl according to the following protocols: 1) Tau/Tau-Cl was added simultaneously with the stimuli for the entire period (24 hours) of cell stimulation (designated protocol A); 2) after 2 hours' pretreatment with indicated concentrations of Tau-Cl, the culture medium was discarded and fresh medium supplemented with the stimuli was added for another 24 hours (protocol B); 3) after 2 hours' pretreatment with Tau-Cl, the culture medium was replaced and cells were cultured for 24 hours, followed by another 24 hours' stimulation with cytokines (protocol C); 4) cells were prestimulated with cytokines for 24 hours, followed by the addition of Tau-Cl and prolongation of culture for an additional 24 hours (protocol D); and 5) cells were prestimulated with cytokines for 24 hours, then the culture medium was removed and replaced with fresh medium supplemented with Tau/Tau-Cl for another 24 hours (protocol E).

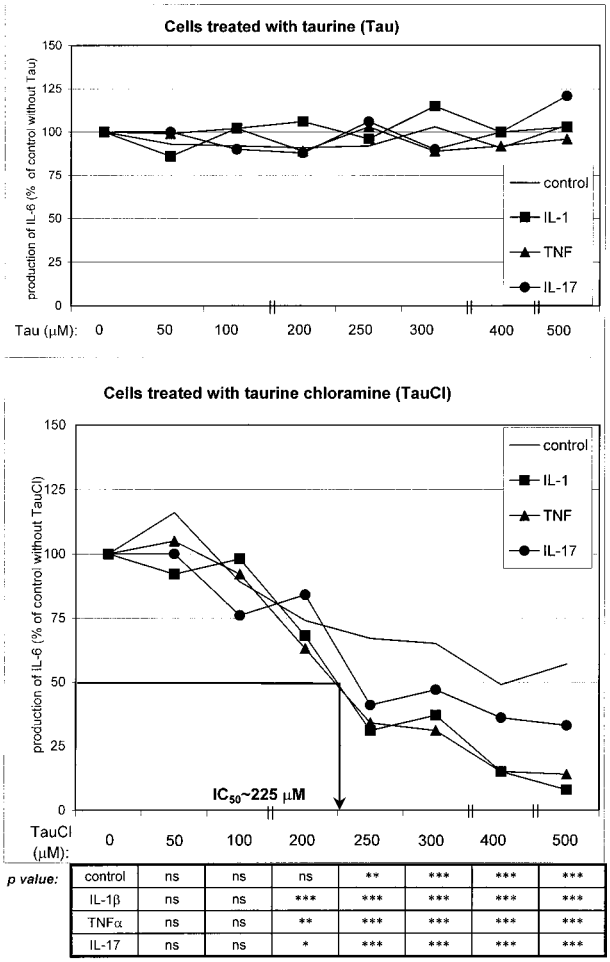
After the above treatments, culture supernatants were collected and clarified by centrifugation (400*g* for 10 minutes), and concentrations of IL-6 and IL-8 were determined by specific ELISAs. The ELISA for IL-6 was done as previously described (25). Briefly, goat polyclonal, neutralizing antibody (Ab) specific for human IL-6 (R&D Systems) was used as the capture Ab, while IL-6–specific rabbit polyclonal Ab (Sigma) was applied as the detection Ab, followed by horseradish peroxidase–conjugated goat anti-rabbit immunoglobulins and *o*-phenylenediamine dihydrochloride (OPD) (both from Sigma) as a substrate. The ELISA for IL-8 was performed using Ab specific for human IL-8 from R&D Systems, according to the manufacturer's protocol. Briefly, mouse monoclonal Ab was used as the capture Ab, while rabbit polyclonal biotinylated Ab was applied as the detection Ab. Streptavidin-peroxidase conjugate (Jackson ImmunoResearch, West Grove, PA), followed by OPD, was added to develop the enzymatic reaction.

Human recombinant cytokine standards were from R&D Systems. The standard curves were determined in culture medium containing 10% FCS. Optical density was measured at 492 nm using an automatic ELISA reader (LP 400; Diagnostics Pasteur, Marnes-La-Coquette, France). The detection limit was 15 pg/ml for IL-6 and 4 pg/ml for IL-8.

Cell proliferation assay. Proliferation of the cells was determined by an assessment of DNA synthesis in FLS, based on the incorporation of tritiated thymidine. For the assay, 96-well, flat-bottom culture plates were seeded with 5×10^3 cells in 0.2 ml of culture medium, recombinant human basic fibroblast growth factor (rHubFGF; R&D Systems) was added to the concentration of 1 ng/ml, and the cells were cultured for 72 hours. Tau and Tau-Cl were added simultaneously with bFGF at the beginning of the culture. ^{3}H-methyl-thymidine (2 μCi/ml; Amersham, Buckinghamshire, UK) was added 18 hours before the termination of cell culture. Cells were collected from the culture plates using cell harvester (Skatron, Lier, Norway), and radioactivity of the samples was measured using liquid scintillation counter (1209 Rackbeta; Pharmacia, Uppsala, Sweden).

Viability of the cells. In every experiment, the viability of cells, assayed by trypan blue exclusion, was controlled. In the cultures of both untreated and cytokine-stimulated FLS, the cell viability was ≥95% and the cells kept fibroblast morphology. Tau (50–500 μM) affected neither the viability nor the morphology of the cells. In contrast, in the presence of high concentrations of Tau-Cl (400–500 μM), cells changed morphology (~30–50% of the cells became round-shaped and detached from the plastic surface). However, even at this high concentration of Tau-Cl, cell viability was ≥95%.

Statistical analysis. Repeated-measures analysis of variance, followed by Tukey's test, was applied to evaluate the effect of stimuli and Tau/Tau-Cl. All data are expressed as the mean ± SEM. Probability values less than 0.05 were considered to be statistically significant.



p value:								
control	ns	ns	ns	**	***	***	***
IL-1β	ns	ns	***	***	***	***	***
TNFα	ns	ns	**	***	***	***	***
IL-17	ns	ns	*	***	***	***	***

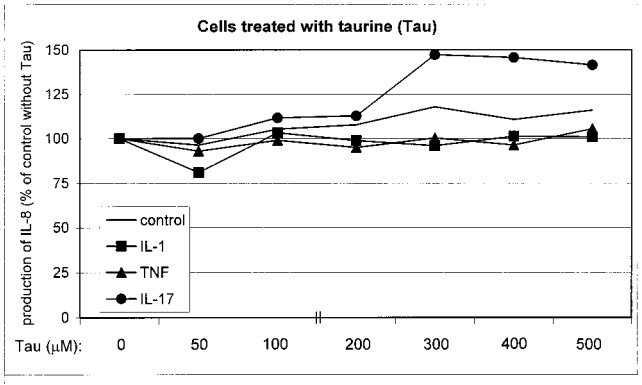
Figure 2. Effects of taurine (Tau) or taurine chloramine (Tau-Cl) on the production of IL-6 by FLS of RA patients. Cells (2×10^4/ml) were stimulated for 24 hours with recombinant human IL-1β (1 ng/ml), TNFα (10 ng/ml), or IL-17 (10 ng/ml) in the presence of different concentrations of Tau or Tau-Cl. The control represents nonstimulated cells. The concentration of IL-6 was determined in culture supernatants by enzyme-linked immunosorbent assay. Results are expressed as a percentage of the responses noted in the control cell cultures, which were treated with neither Tau nor Tau-Cl. Values are the mean of 19 experiments (non-stimulated cells, IL-1β– and TNFα-stimulated cells) or 10 experiments (IL-17–stimulated cells). Differences between the cells treated with Tau and untreated cells were not significant. The arrow indicates the concentration of Tau-Cl that caused 50% inhibition of the cytokine-induced IL-6 response (IC_{50} value ~225 μM of Tau-Cl). * = P = 0.01–0.001; ** = P = 0.001–0.0001; *** = P = 0.0001–0.00001 for Tau-treated versus Tau-Cl–treated cells. ns = not significant (see Figure 1 for other definitions).

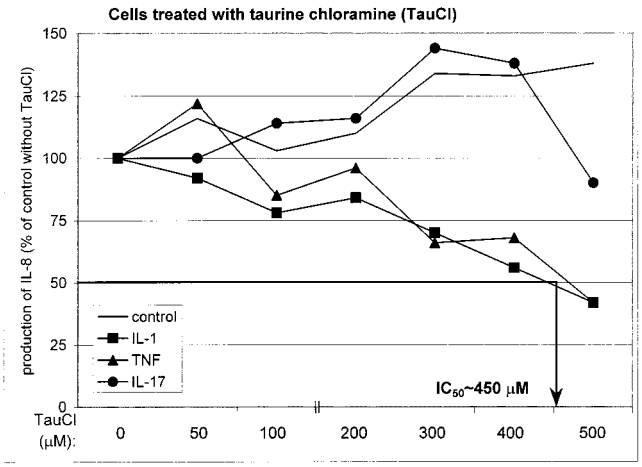
RESULTS

Cytokine-triggered synthesis of IL-6 and IL-8 by RA FLS. As shown in Figure 1, the cells spontaneously secreted a minute amount of both IL-6 (mean ± SEM 502 ± 72 pg/ml; n = 19) and IL-8 (299 ± 103 pg/ml; n = 19). In the presence of exogenously added cytokines (IL-1β, TNFα, or IL-17), the secretion of IL-6 (mean ± SEM 9.3 ± 0.9, 3.2 ± 0.4, or 1.3 ± 0.25 ng/ml, respectively) and IL-8 (11.7 ± 2.1, 7.4 ± 0.8, or 0.67 ± 0.17 ng/ml, respectively) by these cells rose significantly (Figure 1). Interestingly, the magnitude of both IL-6 and IL-8 secretion showed a similar dependency on the type of stimulus: IL-1β (19- and 39-fold increase, respectively) > TNFα (6- and 25-fold increase, respectively) > IL-17 (3- and 2-fold increase, respectively).

Effects of Tau and Tau-Cl on the synthesis of IL-6 and IL-8 by RA FLS. In the first part of the present study, the effects of Tau or Tau-Cl on the production of IL-6 and IL-8 were evaluated by addition of every compound simultaneously with the stimuli at the onset of cell culture. The levels of spontaneous and cytokine-triggered secretion of IL-6 and IL-8 are presented in Figure 1. Tau had no effect on the spontaneous or cytokine-triggered secretion of either IL-6 (Figure 2) or IL-8 (Figure 3); the differences between the untreated cells and the cells treated with Tau were not statistically significant. In comparison with Tau, Tau-Cl significantly reduced production of both cytokines in a dose-dependent manner (Figures 2 and 3). The spontaneous production of IL-6 was significantly inhibited in the presence of 250–500 μM of Tau-Cl, with the maximal effect (~50% inhibition) observed at 400 μM (Figure 2). The cytokine-induced production of IL-6 was inhibited even more potently by the same range of Tau-Cl concentrations. The level of inhibition of IL-6 secretion by Tau-Cl did not differ significantly between the cells stimulated with IL-1β, TNFα, or IL-17, and the 50% inhibitory concentration (IC_{50}) was calculated to be similar (~225 μM) (Figure 2).

In contrast to IL-6 production, neither spontaneous nor IL-17–induced secretion of IL-8 was affected by Tau-Cl (Figure 3). Although Tau-Cl at concentrations of >200 μM showed a tendency to elevate spontaneous and IL-17–triggered IL-8 secretion, the differences were not significant in comparison with Tau-treated cells. Importantly, both TNFα- and IL-1β–triggered IL-8 responses were inhibited with similar potency by high 400–500 μM concentrations of Tau-Cl, with an IC_{50} value of ~450 μM (Figure 3). It should be emphasized that similar inhibitory effects of Tau-Cl were noted when the concentrations of IL-6 and IL-8 were determined intracellularly, in the cell lysates (data not shown). The latter results suggest that the inhibitory action of Tau-Cl is not restricted to secretion, but also influences the synthesis of IL-6 and IL-8 in RA FLS.





p value:						
control	ns	ns	ns	ns	ns	ns
IL-1β	ns	ns	ns	ns	*	**
TNFα	ns	ns	ns	ns	*	**
IL-17	ns	ns	ns	ns	ns	ns

Figure 3. Effects of Tau or Tau-Cl on the production of IL-8 by FLS of RA patients. Differences between the cells treated with Tau and untreated cells were not significant. The arrow indicates the concentration of Tau-Cl that caused 50% inhibition of the TNFα- or IL-1β–triggered IL-8 response (IC_{50} value ~450 μM of Tau-Cl). * = P = 0.01–0.001; ** = P = 0.001–0.0001 for Tau-treated versus Tau-Cl–treated cells. See Figures 1 and 2 for definitions.

Differing inhibition of IL-6 and IL-8 production according to the time of Tau-Cl addition. In the next part of the study, the cells were stimulated for 24 hours with IL-1β (1 ng/ml), but treated differently with Tau-Cl (Figure 4, bars A–E). In control cell cultures, Tau-Cl was replaced with complete culture medium. Both the spontaneous and IL-1β–triggered secretion of IL-6 (~500 pg/ml and ~10 ng/ml, respectively) and IL-8 (~250 pg/ml and ~13 ng/ml, respectively) did not differ significantly between the protocol groups A–E.

To determine whether the inhibitory effects of Tau-Cl on IL-1β–triggered cytokine production was either short or long-lasting, the following experiments were performed: Tau-Cl was added simultaneously with the stimulus for the entire period of cell stimulation (Figure 4, bar A); cells were briefly pretreated with Tau-Cl (2 hours) and suspended in the fresh medium

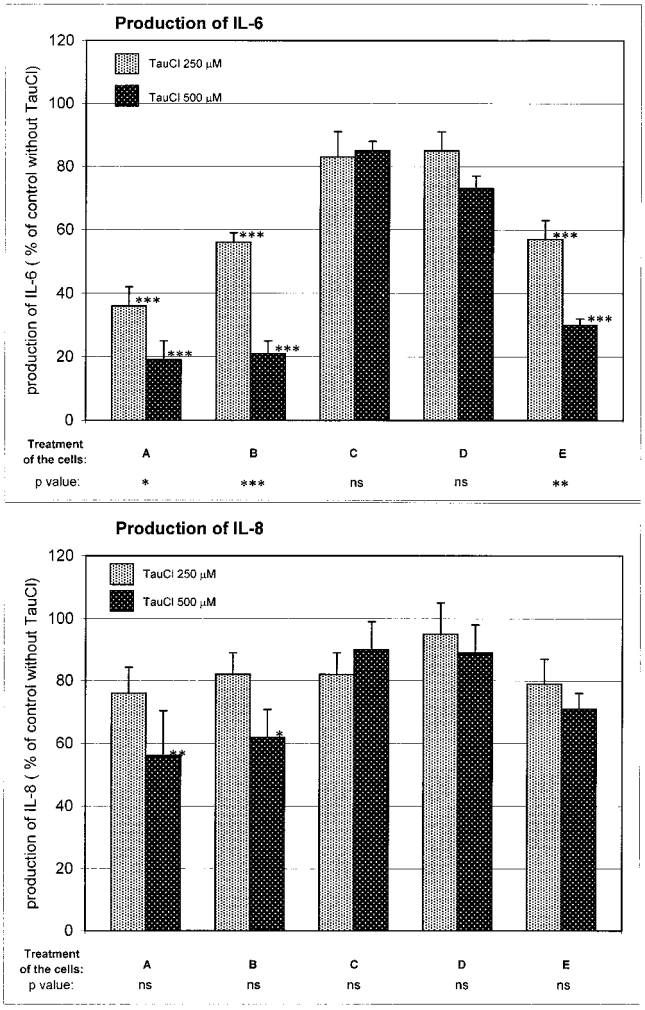


Figure 4. Inhibitory effect of Tau-Cl on IL-6 and IL-8 production dependent on the conditions of cell treatment. Fibroblast-like synoviocytes (2×10^4/ml) were treated with recombinant human IL-1β (1 ng/ml) and Tau-Cl (250 or 500 μM) according to the protocols A–E, as described in Patients and Methods. Concentrations of IL-6 and IL-8 were determined in culture supernatants by enzyme-linked immunosorbent assay. Results are expressed as the percentage of responses noted in the control cell cultures, which were not treated with Tau-Cl. Values are the mean and SEM of 6 experiments. Statistical comparison (P value) between the cells treated with Tau-Cl and untreated cells is indicated with asterisks above the bars, while the differences between the cells treated with 250 μM or 500 μM of Tau-Cl are indicated with asterisks below the x-axis. * = P = 0.05–0.01; ** = P = 0.01–0.001; *** = P = 0.001–0.0001. ns = not significant (see Figures 1 and 2 for definitions).

devoid of Tau-Cl, and the stimulus was added either immediately (Figure 4, bar B) or after 24 hours of culture of the cells (Figure 4, bar C). According to the respective IC_{50} values (Figures 2 and 3), the production of IL-6 was significantly inhibited in the presence of either 250 μM or 500 μM of Tau-Cl, while IL-8 was affected only by 500 μM of Tau-Cl (Figure 4).

Similar inhibitory effects on the production of IL-6 (~80% inhibition) and IL-8 (~40% inhibition) were observed when Tau-Cl was added at the time of cell activation (Figure 4, bar A) or 2 hours before stimulus (Figure 4, bar B). In contrast, pretreatment of the cells with Tau-Cl was not sufficient to inhibit the production of IL-6 and IL-8 if the stimulus was added 24 hours later (Figure 4, bar C). These results show that Tau-Cl may interfere with early events (e.g., signal transduction, transcription of messenger RNA [mRNA]) that regulate production of IL-6 and IL-8.

Because FLS in the inflamed joints of RA patients are continuously exposed to cytokines (e.g., IL-1β, TNFα), we also investigated the effect of Tau-Cl on the production of IL-6 and IL-8 by the cells prestimulated with IL-1β for 24 hours (Figure 4, bars D and E). The addition of Tau-Cl (250 μM or 500 μM) significantly decreased the production of IL-6 only when the stimulus was washed out (Figure 4, bar E), while it was ineffective when the stimulus was still present in the culture medium (Figure 4, bar D). A similar tendency was observed for IL-8 secretion; however, the effect of Tau-Cl on the production of IL-8 by prestimulated cells was not significant (Figure 4, bars D and E). Therefore, these results suggest that in addition to early events, some late events controlling at least the production of IL-6 are also affected by Tau-Cl. Moreover, these results indicate that the inhibitory effect of Tau-Cl on IL-6 and IL-8 production are linked neither to theoretically possible Tau-Cl–induced cytotoxicity (Figure 4, bar C) nor to the reactivity of Tau-Cl with the cytokines present in the culture medium (Figure 4, bars C and D).

Therefore, we conclude that Tau-Cl indeed down-regulates the production of IL-6 and IL-8 by RA FLS. It should be noted that exactly the same results were obtained using FLS treated with TNFα (data not shown), supporting the hypothesis that Tau-Cl may interfere with a common signal transduction pathway.

Tau-Cl inhibition of proliferation of RA FLS. To determine whether Tau or Tau-Cl exerted any effect on the proliferation of the cells, both compounds were added at the beginning of cell culture. The rate of spontaneous proliferation of the cells was low (mean ± SEM 368 ± 75 counts per minute), while bFGF increased it significantly (1,494 ± 436 cpm; $P = 0.04$). Results, presented in Figure 5, show that Tau had no significant effect on either spontaneous or bFGF-triggered cell proliferation. In comparison with Tau, Tau-Cl (500 μM) decreased spontaneous cell proliferation by 57%, while both 250 μM and 500 μM Tau-Cl inhibited bFGF-induced proliferation of these cells by 67% and 85%, respectively.

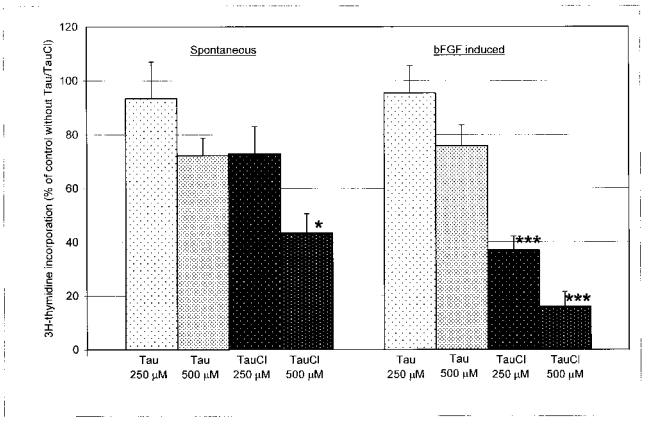


Figure 5. Effect of Tau and Tau-Cl on the proliferation of FLS of RA patients. Cells (5×10^3/well) were cultured for 3 days in culture medium (spontaneous) or in medium supplemented with 1 ng/ml of recombinant human basic fibroblast growth factor (bFGF) in the presence (250 μM or 500 μM) or absence of either Tau or Tau-Cl. ^{3}H-thymidine (1 μCi/well) was added 18 hours before termination of the culture, and cell-bound radioactivity was measured as described in Patients and Methods. Results are expressed as the percentage of responses noted in the control cell cultures that were treated with neither Tau nor Tau-Cl and are the mean and SEM of 5 experiments. * = $P = 0.05$–0.01; *** = $P = 0.001$–0.0001 for Tau-treated versus Tau-Cl–treated cells. See Figures 1 and 2 for other definitions.

DISCUSSION

The results of the present study correspond to previous data showing that FLS of RA patients secrete IL-6 and IL-8 in vitro upon stimulation not only with the cytokines derived from intimal lining macrophages (IL-1β, TNFα), but also when exposed to the lymphokine originated from CD4+ T cells that infiltrate joint tissue (IL-17). However, the latter response is much weaker (3,4) (Figure 1). Moreover, in accordance with the opinion that bFGF supports growth of these cells (5), we noted a significant increase in RA FLS proliferation triggered by this factor (Figure 5).

The present results demonstrate, for the first time, that Tau-Cl inhibits some proinflammatory and pathogenic functions of RA FLS, i.e., the synthesis of

IL-6 and IL-8 (Figures 2 and 3) and cell proliferation (Figure 5). Moreover, we have shown that Tau-Cl exerts an inhibitory effect on the function of these cells at concentrations that are physiologically relevant.

Tau, a precursor of Tau-Cl, is a dominant free amino acid that is present in the millimolar range in most mammalian tissues and human blood cells (10–20 m*M*), while in the plasma and other physiologic fluids, it reaches concentrations of 50–100 μM (26). The extracellular concentration of chloramines 1 hour after neutrophil activation is ~0.1 m*M,* most of which is thought to be chlorotaurine (27). Tau-Cl is long-lasting, with decomposition <5%/hour at 37°C (27). Thus, it is conceivable that during the inflammatory response, Tau-Cl may accumulate and reach local concentrations in the m*M* range. Cells take up Tau-Cl actively by the β amino acids transport system (21). In contrast with a strong and highly cytotoxic oxidant, HOCl, Tau-Cl shows weaker oxidative abilities and, at physiologic concentrations, does not affect viability of neutrophils, macrophages, and endothelial cells (15). Consistently, the present results showed that Tau-Cl is not toxic to RA FLS.

Furthermore, the results of the present study show that Tau-Cl more potently inhibited production of IL-6 (IC_{50} value ~225 μM) than production of IL-8 (IC_{50} value ~450 μM) (Figures 2 and 3). This difference suggests that, in vivo, locally generated Tau-Cl may also act in a dose-dependent manner and thus modulate the inflammatory response with some specificity, by blocking production of proinflammatory IL-6, while temporarily allowing immune cells to migrate into the site of inflammation due to the presence of the chemokine, IL-8.

It is still unclear which intracellular signal transduction pathway(s) are the target for Tau-Cl. Generally, in neutrophils and macrophages, Tau-Cl inhibits generation of inflammatory mediators independent of the type of stimulus (19,22). Therefore, Tau-Cl may affect a common signaling pathway triggered by various stimuli in different cell types. The present data showing that Tau-Cl down-regulated IL-6 production in TNFα-, IL-1β–, and IL-17–treated cells with the same potency (Figure 2) support this possibility. However, we also show that, in contrast to TNFα- and IL-1β–induced production of IL-8, which was inhibited by Tau-Cl, the synthesis of IL-8 in IL-17–stimulated FLS was not affected in the presence of Tau-Cl (Figure 3). These results indicate that signal transduction pathways triggered by IL-17 and by TNFα/IL-1β may differ.

In agreement with published data (19,20), the present results show that Tau-Cl exerted the strongest inhibitory effect on the production of inflammatory mediators when added simultaneously with the stimuli (Figure 4). Consistently, Tau-Cl has been shown to inhibit transcription of some genes (e.g., encoding inducible nitric oxide synthase) (20,22,28,29), and to block translation of others (e.g., TNFα, cyclooxygenase 2) (28,30). The effect of Tau-Cl on the transcription of IL-6 and IL-8 mRNA in RA FLS is currently being tested in our laboratory. Interestingly, the present results suggest that Tau-Cl may also affect late events that govern IL-6 synthesis in these cells. This assumption is based on our results showing that Tau-Cl decreased cytokine production in prestimulated cells only if the stimulating agent was removed from cell culture (Figure 4; compare bars D and E). The late events that are influenced by Tau-Cl need further study. However, it should be pointed out that Tau-Cl was reported to down-regulate the synthesis of TNFα in macrophages (19,21). Thus, it is likely that the effects of Tau-Cl in vivo reflect not only the direct inhibition of IL-6 and IL-8 production, but also the indirect effects due to a lower concentration of TNFα.

Our results demonstrating that Tau-Cl inhibited proliferation of RA FLS draw special attention. Expansion of FLS leads to hyperplasia of RA synovial membrane. The high potential of these cells to grow seems to result from their resistance to apoptotic death (2,12). Therapeutic approaches that focus on enhancing apoptosis and/or diminishing proliferation of RA synoviocytes are theoretically rational, but have not been undertaken yet. Interestingly, results of in vitro studies revealed that PDGF-induced growth of RA synovial fibroblasts is blocked by a macrolide immunosuppressant, rapamycin (31). Moreover, antisense oligonucleotides targeting proliferating cell nuclear antigen also inhibited IL-1–stimulated proliferation of these cells (32). Our new finding that both spontaneous and bFGF-triggered proliferation of these cells was successfully inhibited by a physiologic agent, Tau-Cl (Figure 5), extends the range of potential inhibitors of RA FLS growth. In this context, it is worth mentioning that we observed that at high (400–500 μM), but still not cytotoxic, concentrations, Tau-Cl also decreased the adhesion of RA FLS in vitro (see Patients and Methods). These results are consistent with previous findings showing detachment of cultured myocytes upon exposure to Tau-Cl (33). Although this question needs further detailed study, it is tempting to speculate that Tau-Cl may reduce the invasive behavior of RA FLS.

There is evidence that Tau, either directly or indirectly (via Tau-Cl), exerts antiinflammatory effects in vivo. Oral administration of Tau was reported to

prevent or attenuate lung inflammation in animals (34–36). In humans, the preoperative administration of Tau in order to prevent patients from neutrophil-mediated vascular leak syndrome has been proposed (37). In psoriasis, a decreased level of neutrophil Tau was reported (38). Since Tau, probably through the activation of the neutrophil myeloperoxidase-H_2O_2-halide system, inhibits in vitro generation of leukotriene B_4 (LTB_4) by these cells (37,39), the use of Tau and possibly Tau-Cl in order to normalize LTB_4 in psoriatic patients is now under consideration. Regarding RA patients, data showing altered functions of synovial fluid neutrophils (16), the presence of antineutrophil cytoplasmic antibodies which may influence neutrophil functions (40), and the elevated plasma level of Tau (17), as well as hypertaurinuria (18) suggest the disturbed metabolism of Tau/Tau-Cl and point out the importance of further studies on this subject.

In summary, the present results show, for the first time, that, in vitro, Tau-Cl inhibits several pathogenic functions (the synthesis of IL-6 and IL-8, and cell proliferation) of RA FLS. Whether Tau-Cl exerts the same activity in vivo requires further study on animal models of RA. Although it is premature to predict the results of these studies, it is tempting to speculate that future application of Tau-Cl in human therapy could be based on the administration of either Tau (to supplement hypophysiologic levels of this amino acid in patients with normal functioning neutrophils) or Tau-Cl (in patients with a disturbed neutrophil myeloperoxidase-H_2O_2-halide system).

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