The effect of taurine chloramine on pro-inflammatory cytokine production by peripheral blood mononuclear cells isolated from rheumatoid arthritis and osteoarthritis patients

M. Chorąży-Massalska[1], E. Kontny[1], A. Kornatka[1], M. Rell-Bakalarska[2], J. Marcinkiewicz[3], W. Maśliński[1]

[1]Department of Pathophysiology and Immunology, [2]Outpatient's Clinic, Institute of Rheumatology, Warsaw, Poland; [3]Department of Immunology, Jagiellonian University Medical College, Cracow, Poland.

Abstract

Objective

Pro-inflammatory cytokines play a critical role in the pathogenesis of RA. A natural oxidant, TauCl exerts anti-inflammatory activities. Here, the effects of Tau and TauCl on key pro-inflammatory cytokines – IL-1β, IL-6 and TNF-α production by LPS-triggered peripheral blood mononuclear cells (PBMCs) isolated from RA and OA patients and healthy blood donors – were examined.

Methods

PBMCs were stimulated with LPS (24 h) in the presence of Tau or TauCl (200-400 μM). Cytokine production was measured in culture supernatants (secreted) and cells lysates (cell-associated) using specific ELISAs.

Results

Production of the secreted forms of IL-1β and IL-6 was inhibited by TauCl with IC_{50} ≈250 μM and 300-400 μM respectively, in all investigated groups. In all cultures of PBMCs TauCl raised the TNF-α production at the low concentration (200 mM), while at the higher concentration (400 μM) either reduced it (55% of RA, 70% of OA patients and 55% of healthy donors) or exerted no effect (remainder of patients). Interestingly, Tau did not significantly affect any cytokine production.

Conclusion

TauCl at high concentrations down-regulates pro-inflammatory cytokine production. However, the impact of TauCl on TNF-α production by PBMCs from RA is more limited than in cells isolated from OApatients.

Key words

Taurine chloramine (TauCl), pro-inflammatory cytokines, peripheral blood mononuclear cells (PBMCs).

Magdalena Chorąży-Massalska, MSc; Ewa Kontny, PhD; Anna Kornatka; Maria Rell-Bakalarska, MD; Janusz Marcinkiewicz, Professor; Włodzimierz Maśliński, Professor.

Please address correspondence to: Ewa Kontny, PhD, Department of Pathophysiology and Immunology, Institute of Rheumatology, Spartanska 1, 02-637 Warsaw, Poland.
E-mail: zpatiir@warman.com.pl

Received on February 4, 2004; accepted in revised form on July 16, 2004.

Introduction

The role of pro-inflammatory cytokines, produced either by cells infiltrating inflammed joints or by fibroblast-like synoviocytes (FLS), is well established and documented in RA pathogenesis (1, 2). Among a broad array of cytokines detected in inflammed joints (3) IL-1β, IL-6 and TNF-α appear to be of special importance. These cytokines actively induce and regulate both innate and acquired immune responses (4) and their blood concentration correlates with the severity of RA (1). Further support for the participation of these cytokines in pathogenic processes comes from the effectiveness of therapies based on the modulation or blocking activities of TNF-α (5), IL-1β (6) and IL-6 (7) in RApatients.

Two major cell types infiltrating RA synovium are neutrophils in the synovial fluid and macrophages in the synovial tissue (1,8). It is well known that release of reactive oxidants from these cells in inflammed joints is at least partly responsible for the tissue damage described in RA(9). Hypochlorous acid (HOCl) is generated mostly by neutrophils and represents the major product of their oxidative metabolism. Because of its high reactivity, HOCl is immediately consumed in many reactions, e.g. with amines that yield additional new oxidants (10). Taurine, an abundant amino acid present in most mammalian tissues, is a well known antioxidant. Taurine chloramine formed in the reaction of Tau with HOCl, is a long-lived, but weaker oxidant than HOCl endowed with many anti-inflammatory properties (11).

In the light of growing evidence regarding the anti-inflammatory role of taurine chloramine in general and especially against the pathological properties of FLS (12), we were interested in whether TauCl also affects the production of pro-inflammatory cytokines by PBMCs. Since TauCl affects the synthesis of pro-inflammatory cytokines produced by PBMCs isolated from healthy volunteers (13), in the present study we compared the ability of TauCl to modulate the production of pro-inflammatory cytokines (IL-1β, IL-6, TNF-α) by PBMCs isolated from RAand OApatients.

Materials and methods

Patients and control subjects

Thirty patients with RA or OA symptoms were recruited from the Outpatient Clinic at the Institute of Rheumatology. All patients met the American College of Rheumatology classification criteria for RA (n=20), or OA (n=10) (14). The study was approved by the local Ethics Commitee. Most of the RA patients (85%) were receiving a slow-acting anti-rheumatic drug sulfasalazine (14 patients) or sulfasalazine plus encorton (3 patients) and all of the OA patients were being treated with nonsteroid anti-inflammatory drugs (NSAID). To better understand the correlation between the effect of TauCl treatment on production of pro-inflammatory cytokines, we divided the RA patients into 2 groups: younger (9F/1M, age range 25-49 years, mean ± SEM = 40,0 ± 3,0) and older (9F/1M, age range 56-79 years, mean ± SEM = 65.7±1.9) than 50. For comparison, the production of pro-inflammatory cytokines were measured in 20 age-matched healthy volunteers: 10 younger (6F/4M, age range 20-49 years, mean± SEM=35.0±3.3) and 10 older (7F/3M, age range 52-75 years, mean ± SEM = 61.6±2.1) than 50 years. The OA patients (10F, age range 50-74 years, mean ± SEM = 63.9 ± 2.8) were considered as an older group.

Cell culture and treatment

PBMCs were isolated from heparinized blood on Gradisol L density gradient (Polfa, Poland). Cells (1 x 10^6/ml) were cultured in 24-well flat-bottom plates (Nunc A/S, Roskilde, Denmark) in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS; Biochrom KG, Berlin, Germany), 2 mM L-glutamine, 100 units/ml penicillin, 10 μg/ml kanamycin and 100 μg/ml streptomycin in humidified 5% CO_2 atmosphere at 37ºC. Cells, obtained from each donor, were stimulated for 24 hours with 5 μg/ml lipopolysaccharide (LPS; *E. coli* 055:B5; Difco, Detroit, MI) in the presence of either Tau/TauCl or control media. TauCl or Tau was added at the 200-400 μM concentration simultaneously with the activator. Neither compound was cytotoxic at the

tested concentrations, as estimated by the measurement of lactate dehydrogenase activity (LDH; Takara Shuzo Co, Otsu, Shiga, Japan) in culture supernatants. After incubation, the supernatants were collected, clarified by centrifugation at 400xg for 10 minutes and frozen at -70ºC until the assay estimating the production of the secreted form of the cytokines. The synthesis of the cell-associated form of cytokines was measured in cell lysates, obtained by 3 cycles of freezing-thawing of cells resuspended in 1 ml of freshly added culture medium.

ELISAs for IL-1β, IL-6 and TNF-α detection

Sandwich ELISAs for measurement of IL-1β and IL-6 concentrations were carried out using the modified method described by Kontny *et al.* (15). Goat polyclonal antibody (Ab) specific for human IL-6 and mouse monoclonal anti-human IL-1β Ab (both from R&D Systems, Minneapolis, MN) were used as the capture antibodies, respectively. Cytokine specific rabbit polyclonal Abs (both from Sigma, St. Louis, MO, USA) were used as the detection antibodies, followed by horseradish peroxidase-conjugated goat anti-rabbit Ab and o-phenylenediamine dihydrochloride (Sigma) as substrate. Recombinant human IL-1β and IL-6 (R&D Systems) were used as standards. The TNF-α concentration was specifically quantified using Opt EIA™ S_{ET} (Pharmingen, San Diego, CA). Optical density was determined with an ELISA reader (LP 400, Diagnostics Pasteur, France) at 450 nm (for TNF-α) and 492 nm (for IL-1β and IL-6). The sensitivity limit was 15.6 pg/ml for TNF-α and 39 pg/ml for both IL-1β and IL-6.

Synthesis of TauCl

Taurine monochloramine was prepared by chlorination of taurine (Sigma) with NaOCl (Aldrich, Steinheim, Germany) using the method previously described (16). Stock solutions of Tau and TauCl were kept at 4ºC for a maximum period of 3 days before use.

Statistical analysis

Data are presented as the mean ± SEM. Repeated measures analysis of variance (ANOVA) followed by post hoc Tukey's test were applied to evaluate the effect of Tau/TauCl on LPS stimulated cells. P values less than 0.05 were considered significant.

Results

Spontaneous production of IL-1β, IL-6 and TNF-α in RA and OA patients and healthy volunteers

Spontaneous production of IL-6 and TNF-α by PBMCs isolated from healthy volunteers but not from RA patients, increased with age showing a positive correlation (data not shown). Interestingly, there was a tendency toward the higher spontaneous production of IL-1β, IL-6 and TNF-α by RA patients younger than 50 years (mean ± SEM = 615.2 ±334.4; 5903.8 ± 2210.0; 640.1 ±418.2 pg/ml respectively) than in age-matched healthy donors (mean ± SEM = 75.0 ± 49.6; 2707.4 ± 1519.4; 84.7 ± 47.1 pg/ml respectively) (data not shown). LPS significantly raised the synthesis of secreted and cell-associated forms of tested cytokines, resulting in an increase of IL-6> IL-1β > TNF-α secretion (Table I). The majority of produced cytokines was secreted outside the cells. There were no significant differences in spontaneous or LPS-stimulated cytokine production between healthy blood donors and RA or OA patients when compared by age (younger or older than 50 years). The statistically important difference between OA patients and the age-matched healthy group (people belonging to both groups were older than 50 years) was observed only in the LPS-induced production of IL-6 (18876 vs 11982 pg/ml, $p < 0.05$).

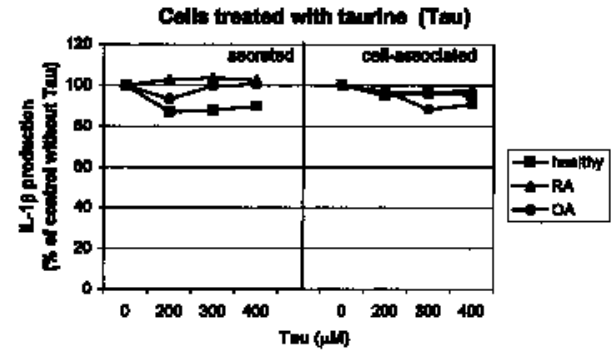
The effect of taurine and taurine chloramine on IL-1β, IL-6 and TNF-α production by PBMCs isolated from RA, OA patients and healthy controls

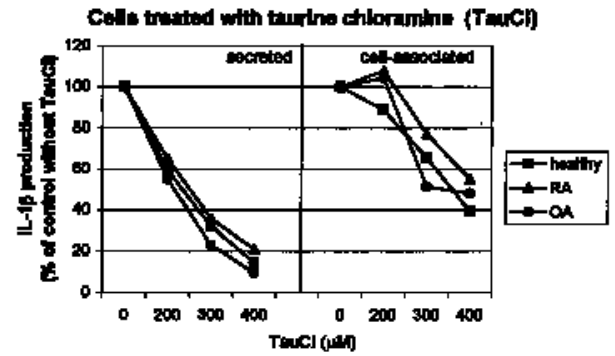
Taurine did not exert a significant effect on the LPS-triggered production of either IL-1β (Fig. 1) or IL-6 (Fig. 2) or TNF-α (data not shown) in any of the investigated groups. In contrast, TauCl significantly and in a dose-dependent manner reduced LPS-induced cytokine production of both forms of IL-1β and IL-6 (Figs. 1-2). Production of IL-1β was inhibited by a lower concentration of TauCl ($IC_{50} \approx 250$ μM) than IL-6

Table I. The stimulatory effect of LPS on IL-1β, IL-6 and TNF-α production.

Group		IL-1β (pg/ml)		IL-6 (pg/ml)		TNF-α (pg/ml)	
		Secreted	Cell-associated	Secreted	Cell-associated	Secreted	Cell-associated
Healthy	C vs LPS	659,5 vs 3903,8	373,4 vs 1252,6	6769,3 vs 19094,3	974 vs 3451	511,6 vs 2312,3	75,2 vs 304,6
	p	****	****	****	****	***	***
RA	C vs LPS	871,1 vs 3155,8	550,8 vs 1059,6	6571 vs 13363,7	630,7 vs 1311,8	448,4 vs 1888,8	48,1 vs 151,5
	p	***	NS	**	**	***	****
OA	C vs LPS	953,2 vs 3175,7	455,9 vs 771,5	7535,9 vs 11981,8	754,4 vs 1208,8	523 vs 1306,1	80,5 vs 167,2
	p	***	NS	*	NS	**	*

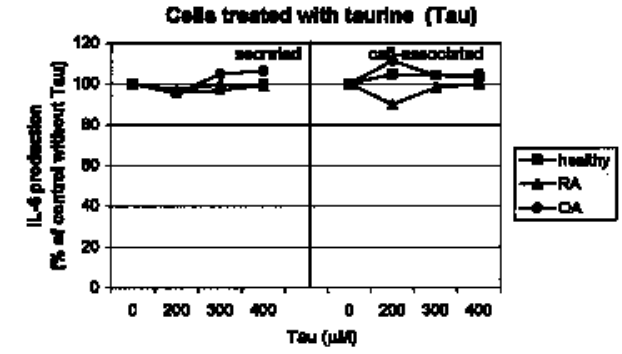
C: not stimulated; LPS: after LPS stimulation; p value ≤ 0,05 were considered as statistically significant *0.01<P< 0.05; **0.001 < P < 0.01; ***0.0001<P< 0.001; ****0.00001< P< 0.0001; NS: not significant.

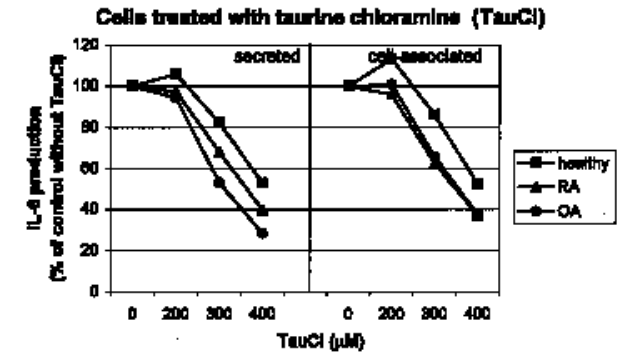




TauCl concentration (μM):			200	300	400
P value:	healthy	S	***	***	***
		C	NS	***	***
	RA	S	****	****	****
		C	NS	NS	****
	OA	S	**	***	***
		C	NS	NS	*

Fig. 1. Effects of taurine (Tau) or taurine chloramine (TauCl) on the LPS triggered production of IL-1β by peripheral blood mononuclear cells (PBMCs). Cells were cultured 24 hours with 5mg/ml LPS (control) or in the presence of investigated concentrations of Tau or TauCl. Concentrations of two forms of the cytokine: secreted in culture supernatants (**S**) and cell-associated in lysates (**C**) were measured by ELISAspecific for IL-1β (as described in Materials and Methods). Results are expressed as a percentage of the responses noted in cell cultures without Tau or TauCl and represent the cytokine production by PBMCs isolated from peripheral blood of RA (n = 20), OA (n = 10) patients and healthy volunteers (n = 20). The differences between control cells and those treated with Tau were statistically insignificant. *0.01 < P< 0.05; **0.001 < P< 0.01; ***0.0001 < P< 0.001; ****0.00001 < P < 0.0001; for TauCl treated versus control cells. NS = not significant.





TauCl concentration (μM):			200	300	400
P value:	healthy	S	NS	*	***
		C	NS	NS	***
	RA	S	NS	****	****
		C	NS	****	****
	OA	S	NS	***	***
		C	NS	NS	*

Fig. 2. Effects of Tau or TauCl on the LPS triggered production of IL-6 by PBMCs. Concentrations of two forms of the cytokine: secreted in culture supernatants (**S**) and cell-associated in lysates (**C**) were measured by ELISA specific for IL-6 . Results are expressed as a percentage of the responses noted in cell cultures without Tau or TauCl and represent the cytokine production by PBMCs isolated from peripheral blood of RA, OA patients and healthy donors. The differences between control cells and those treated with Tau were statistically insignificant. *0.01 < P < 0.05; ***0.0001 < P< 0.001; ****0.00001 < P< 0.0001 for TauCl treated versus control cells. NS = not significant (see Fig. 1 for other definitions).

(IC_{50} ≈300-400 μM) in all investigated groups (Figs. 1-2). The effect of TauCl on TNF-α production was more complex. At the low concentration (200 μM) an enhancement of TNF-α production was observed. At the higher concentration (400 μM) TNF-α production was lowered in some RA and OA patients and in some healthy donors, while in the remaining subjects levels were unchanged. To investigate this finding, all patients and healthy individuals were divided into two groups. Group I consisted of responders, i.e. individuals whose PBMCs produced less TNF-α in the presence of the high concentration (400 μM) of TauCl, and group II (non-responders), whose cells produced equal amount of TNF-α in the presence or absence of TauCl (400 μM) (Table II). In the responder group TauCl decreased TNF-α production with IC_{50} ≈325-375 μM (Fig. 3).

Young RA patients with relatively short disease duration respond to taurine chloramine treatment

More than 50% of the RA, OA patients and healthy controls were considered as responders (Table II). There was no significant difference in age between OA responder and non-responder groups (mean ± SEM = 64.6 ± 3.3 versus 62.3 ± 6.9) or in the mean duration of symptoms (mean ± SEM = 4.4 ± 1.2 versus 3.7 ± 0.9 years). Similarly, heal-

Table II. Patient demographic data.

Diagnosis	Healthy donors (n=20)		RApatients (n=20)		OApatients (n=10)	
	Responders	Non-responders	Responders	Non-responders	Responders	Non-responders
Number of patients (% of all)	11/20 (55%)	9/20 (45%)	11/20 (55%)	9/20 (45%)	7/10 (70%)	3/10 (30%)
Age: mean (range) in years	47,2 (20-75)	49,7 (26-68)	45,9 (25-72)*	61,3 (47-79)*	64,6 (52-73)	62,3 (50-74)
Mean duration of symptoms, years			3,8*	9,0*	4,4	3,7
% older people (> 50 years)	6/11 (55%)	4/9 (44%)	3/11 (27%)	7/9 (78%)	7/7 (100%)	3/3 (100%)
% with short time of symptoms[@]			9/11 (82%)	2/9 (22%)	4/7 (57%)	2/3 (67%)

Footnotes:
RA: rheumatoid arthritis, Oa: osteoarthritis; * Significance (p value ≤ 0,05) responders versus non-responders; no significance was observed between the responder and non-responder groups in healthy donors and OApatients; [@] patients with duration of symptoms ≤ 4 years.

thy responders and non-responders did not differ in age (mean ± SEM = 47.2 ± 5.4 versus 49.7± 4.9). Interestingly, the group of RA responders was much younger than non-responders (mean ± SEM = 45.9 ± 4.8 versus 61.3 ± 3.3; p = 0.01) and were characterized by a shorter duration of symptoms (mean ± SEM = 3.8 ± 1.1 versus 9.0 ± 2.0; p=0.04). 78% of RA non-responders were patients with disease duration more than 4 years and older than 50 years old (Table II). There was no difference between the laboratory parameters (haematocrit, haemoglobin, ESR values; platelets, leukocytes or erythrocytes counts) in the responder versus non-responder groups neither in RAnor OA patients (data not shown).

Discussion

Leukocytes that infiltrate the synovium in RA patients are potent producers of pro-inflammatory cytokines. These cytokines in turn activate FLS (the cells representing active participants of all pathological processes in RA). Data from several laboratories have indicated TauCl, a stable oxidant produced in reaction of taurine with HOCl at the site of inflammation, as an important physiologic immunoregulatory factor (17).

A series of studies performed in our laboratory using fibroblast-like synoviocytes isolated from RA patients, show that TauCl inhibits many pathological functions of RAFLS: their proliferation (18), IL-6 and IL-8 production (19) and COX-2-mediated generation of PGE2 (20). Furthermore, the first study to use TauCl *in vivo* for immune intervention shows that the early administration of TauCl resulted in the delay of the onset of collagen-induced arthritis (CIA) in DBA1/J mice (21).

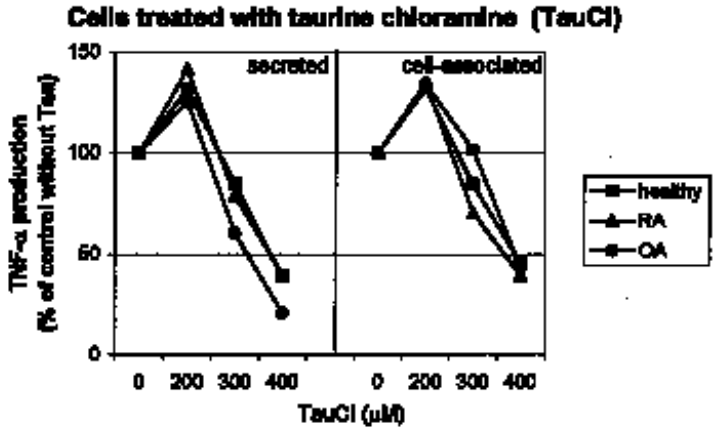
In the present study, the production of IL-1β and IL-6 by human PBMC isolated from RA patients, OA patients and healthy volunteers was significantly reduced in the presence of TauCl. Interestingly, in all investigated groups, TauCl exerted a dual effect on TNF-α production (increasing it at low and reducing at high concentrations). However, significant inhibition of TNF-α synthesis by TauCl was observed only in ≈ 50% of tested patients and healthy donors (the responders group).

The present data, similar to our previously reported findings (13), support the suggestion that TauCl at low concentrations which increase TNF-α synthesis, may favor the initiation of protective inflammation, while at a higher concentration (400 μM) may control the further amplification of the process and gradually reduce pro-inflammatory cytokine synthesis: IL-1β, IL-6 and TNF-α. TauCl by reducing the TNF-α production can limit the level of the Th1 response. This thesis accords with the decreased production of IL-2 by PHA-activated non-adherent leukocytes upon TauCl treatment reported by Park (22). These authors have shown that TauCl, at a concentration of 400 μM, suppressed superoxide anion, IL-6 and IL-8 production in activated healthy human peripheral blood PMNs. However, the reduction in TNF-α in activated monocytes treated with TauCl (400 μM) was not statistically significant. This is in correspondence with present results showing TauCl (400 μM) to be able to reduce significantly the TNF-α production only in some patients belonging to all tested groups. However, according to Marcinkiewicz *et al.* TauCl treatment did not affect the production of IL-2, TNF-α and reactive oxygen species (ROS) by murine dendritic cells (DC)-stimulated T cells (23). Those different effects of TauCl on IL-2 production can result from different cell types or the stimulus used in above mentioned papers.

The physiologic concentrations of TauCl are not known, although taurine is the single most abundant amino acid present in leukocytes (20-50 mM) (17). According to Weiss (24), activation of human neutrophils (~ 2 x 10^6/ml) in the presence of physiologically relevant concentrations of Tau (12.5-15 mM) results in the generation of roughly 100 nmol of TauCl per 2h. Since the inflammatory site is heavily infiltrated with neutrophils (even up to ~25 x 10^6 neutrophils/ml) (10), it is conceivaible that local accumulation of TauCl may easily reach the mM range (12).

Traditionally osteoarthritis, unlike RA, has been treated as a degenerative and non-inflammatory condition. In particular, the participation of cytokines in events leading to RA pathogenesis is widely acknowledged, while in OAclinical indications of inflammation (like synovial swelling and joint effusion) are thought to be a secondary response to tissue injury and not a primary pathogenic event (25). Although OA synovium itself does not exhibit significant cellular proliferation or infiltration by inflammatory leukocytes and OAis not

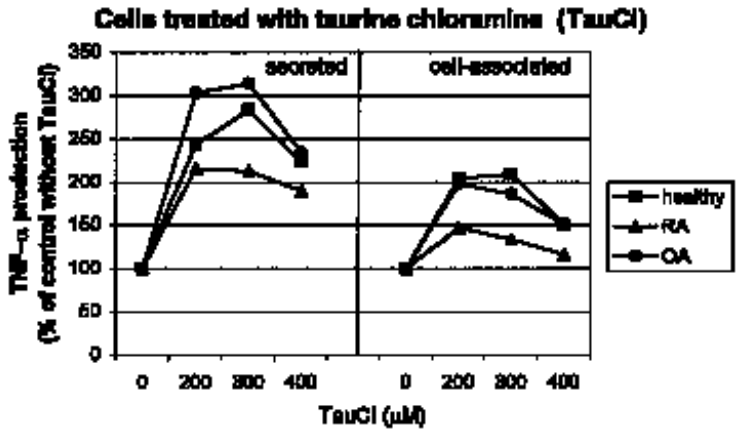
A



Responders group

TauCl concentration (μM):			200	300	400
P value:	healthy	S	*	NS	***
		C	*	NS	***
	RA	S	**	NS	***
		C	*	NS	***
	OA	S	NS	NS	***
		C	NS	NS	NS

B



Non-responders group

TauCl concentration (μM):			200	300	400
P value:	healthy	S	**	***	*
		C	**	**	NS
	RA	S	***	***	***
		C	NS	NS	NS
	OA	S	NS	NS	NS
		C	NS	NS	NS

Fig. 3. Effects of TauCl on the LPS triggered production of TNF-α by PBMCs of responders (**A**) and non-responders (B) groups. Concentrations of two forms of the cytokine: secreted in culture supernatants (S) and cell-associated in lysates (**C**) were measured by ELISAspecific for TNF-α. Results are expressed as a percentage of the responses noted in cell cultures without TauCl and represent the cytokine production by PBMC isolated from peripheral blood of responders (**A**) and non-responders (**B**) for TauCl treatment of RA, OApatients and healthy donors. *0.01 < P< 0.05; **0.001 < P< 0.01; ***0.0001 < P< 0.001; ****0.00001 < P< 0.0001 for TauCl treated versus control cells. NS = not significant (see Fig. 1 for other definitions).

a systemic disease, there is high upregulation of various pro-inflammatory genes in human OAcartilage as compared with normal cartilage (26). In the LPS-induced secretion of IL-6 by PBMCs isolated from healthy people (but only in the group older than 50 years) and OA patients (the whole group is older than 50 years), a statistically important difference was noticed (18876 vs 11982 pg/ml; $p < 0.05$). This would support the notion that the "inflammatory component" is not restricted only to the cartilage and bone and that although OA-affected chondrocytes do not qualify for the typical definition of inflammation, it cannot be treated as “non-inflammatory control” or a surrogate for normal joint tissue. Although the effect of TauCl on OA PBMCs is comparable with that exerted on PBMCs from RAand healthy donors (Figs. 1-3) the percent of responders from this group is much higher (70%) than in RA or healthy people (in both 55%).

Although TauCl triggered the inhibition of TNF-α synthesis by PBMCs isolated from some, but not all healthy donors (55%), and RA (55%) and OA (70%) patients, the RAresponder group was characterized by younger age and shorter duration of symptoms. Thus, it is possible that TauCl is a more potent inhibitor of pro-inflammatory cytokine synthesis in the early than in the late phase of RAdevelopment.

In conclusion, we present evidence that TauCl at concentrations higher than 300 mM inhibits pro-inflammatory cytokine production by peripheral blood mononuclear cells. These anti-inflammatory properties of TauCl may be of therapeutic value.

References

1. BADOLATO R, OPPENHEIM JJ: Role of cytokines, acute-phase proteins, and chemokines in the progression of rheumatoid arthritis. *Semin Arthritis Rheum* 1996; 26: 526-38.
2. FELDMANN M, BRENNAN FM, MAINI RN: Role of cytokines in rheumatoid arthritis. *Annu Rev Immunol* 1996; 14: 397-440.
3. FIRESTEIN GS: Evolving concepts of rheumatoid arthritis. *Nature* 2003; 423: 356-61.
4. DINARELLO CA, MOLDAWER LL: Cytokines regulate the innate response. *In* DINARELLO CA and MOLDAWER LL (Eds.): *Pro-Inflammatory and Anti-Inflammatory Cyto - kines in Rheumatoid Arthritis. A Primer for Clinicians.* CA, Amgen Inc. 1999: 10-16.
5. FELDMANN M, MAINI RN: TNF defined as a therapeutic target for rheumatoid arthritis and other autoimmune diseases. *Nature Medicine* 2003; 9: 1245-50.
6. DAYER J-M: The pivotal role of interleukin-1 in the clinical manifestations of rheumatoid arthritis. *Rheumatology* 2003; 42 (Suppl. 2): ii3-ii10.
7. CHOY EHS, ISENBERG DA, GARROOD T *et*

al.: Therapeutic benefit of blocking interleukin-6 activity with an anti-interleukin-6 receptor monoclonal antibody in rheumatoid arthritis. *Arthritis Rheum* 2002; 46: 3143-50.
8. GRANT EP, PICARELLA D, BURWELL T *et al*.: Essential role for the C5a receptor in regulating the effector phase of synovial infiltration and joint destruction in experimental arthritis. *J Exp Med* 2002; 196: 1461-71.
9. BABIOR BM: Phagocytes and oxidative stress. *Am J Med* 2000; 109: 33-44.
10. WEISS SJ: Tissue destruction by neutrophils. *N Engl J Med* 1989; 320: 365-76.
11. KOPROWSKI M, MARCINKIEWICZ J: Taurine chloramine – its role in immunity and new perspectives for clinical use. *CEJI* 2002; 27: 69-74.
12. KONTNY E, MASLINSKI W, MARCINKIEWICZ J: Anti-inflammatory activities of taurine chloramine. Implication for immunoregulation and pathogenesis of rheumatoid arthritis. *In* LOMBARDINI JB, SCHAFFER SW and AZUMA J (Eds.): *Advances in Experimental Medicine and Biology*. New York, Boston, Dortrecht, London, Moscow, Kluwer Academic/Plenum Publishers 2003: 329-40.
13. CHORAZY M, KONTNY E, MARCINKIEWICZ J, MASLINSKI W: Taurine chloramine modulates cytokine production by human peripheral blood mononuclear cells. *Amino Acids* 2002; 23: 407-13.
14. GABRIELSE: Classification of rheumatic diseases. *In* HOCHBERG MC, SILMAN AJ, SMOLEN JS *et al.* (Eds.): *Reumatology*, 3rd ed., vol. I. Philadelphia, Mosby Elsevier Science 2003: 9-12.
15. KONTNY E, ZIOLKOWSKA M, RYZEWSKA A, MASLINSKI W: Protein-kinase C-dependent pathway is critical for the production of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6). *Cytokine* 1999; 11: 839-48.
16. MARCINKIEWICZ J, GRABOWSKA A, BERETA J, BRYNIARSKI K, NOWAK B: Taurine chloramine down-regulates the generation of murine neutrophil inflammatory mediators. *Immunopharmacology* 1998; 40: 27-38.
17. SCHULLER-LEVIS G, PARK E: Taurine: new implications for an old amino acid. *FEMS Microbiol Lett* 2003; 226: 195-202.
18. KONTNY E, GRABOWSKA A, KOWALCZEWSKI J *et al*.: aurine chloramine inhibition of cell proliferation and cytokine production by rheumatoid arthritis fibroblast-like synoviocytes. *Arthritis Rheum* 1999; 42: 2552-60.
19. KONTNY E, SZCZEPANSKA K, KOWALCZEWSKI J *et al*.: The mechanism of taurine chloramine inhibition of cytokine (interleukin-6, interleukin-8) production by rheumatoid arthritis fibroblast-like synoviocytes. *Arthritis Rheum* 2000; 43: 2169-77.
20. KONTNY E, RUDNICKA W, KOWALCZEWSKI J, MARCINKIEWICZ J, MASLINSKI W: Selective inhibition of cyclooxygenase 2-generated prostaglandin E2 synthesis in rheumatoid arthritis synoviocytes by taurine chloramine. *Arthritis Rheum* 2003; 48: 1551-5.
21. KWASNY-KROCHIN B, BOBEK M, KONTNY E *et al*.: Effect of taurine chloramine, the product of activated neutrophils, on the development of collagen-induced arthritis in DBA1/J mice. *Amino Acids* 2002; 23: 419-26.
22. PARK E, JIA J, QUINN MR, SCHULLER-LEVIS G: Taurine chloramine inhibits lymphocyte proliferation and decreases cytokine production in activated human leukocytes. *Clin Immunology* 2002; 102: 179-84.
23. MARCINKIEWICZ J, NOWAK B, GRABOWSKA A, BOBEK M, PETROVSKA L, CHAIN B: Regulation of murine dendritic cell functions *in vitro* by taurine chloramine, a major product of the neutrophil myeloperoxidase-halide system. *Immunology* 1999; 98: 371-8.
24. WEISS SJ, KLEIN R, SLIVKA A, WEI M: Chlorination of taurine by human neutrophils. *J Clin Investigations* 1982; 70: 598-607.
25. WOLLHEIM FA: Pathogenesis of osteoarthritis. *In* HOCHBERG MC, SILMAN AJ, SMOLEN JS *et al*. (Eds.): *Reumatology*, 3rd ed., vol. II. Philadelphia, Mosby Elsevier Science 2003: 1801-15.
26. ATTUR MG, DAVE M, AKAMATSU M, KATOH M, AMIN AR: Osteoarthritis or osteoarthrosis: the definition of inflammation becomes a semantic issue in the genomic era of molecular medicine. *Osteoarthritis Cart* 2002; 10: 1-4.