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Impaired generation of taurine chloramine by synovial fluid neutrophils of rheumatoid arthritis patients

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Summary. Taurine (Tau), a dominant free amino acid present in neutrophil cytoplasm, serves as a scavenger for hypochlorous acid (HOCl) released during these cells activation. The resulting taurine chloramine (Tau-Cl) exerts potent anti-inflammatory properties. In the present study we tested the hypothesis that the formation of Tau-Cl is impaired in neutrophils isolated from rheumatoid arthritis (RA) patients. The inhibition of zymosan-triggered chemiluminescence in the presence of exogenous Tau was used for indirect measurement of Tau-Cl generation. The chemiluminescence of neutrophils isolated from peripheral blood (PB) of healthy volunteers and RA patients was inhibited by Tau with similar potency. By contrast, synovial fluid (SF) neutrophils of these patients were significantly less sensitive for Tau-mediated inhibition. Therefore, our data indicate impaired generation of Tau-Cl in neutrophils isolated from SF of RA patients.

Keywords: Rheumatoid arthritis – Synovial fluid neutrophils – Taurine chloramine

Abbreviations: Tau, taurine; Tau-Cl, taurine chloramine; PB, peripheral blood; SF, synovial fluid; RA, rheumatoid arthritis

Introduction

Rheumatoid arthritis is an autoimmune disease characterised by chronic synovitis with ensuing destruction of cartilage and bone (Arend, 2001). There is ample evidence that neutrophils contribute to RA pathogenesis. Primed and activated neutrophils: (*i*) accumulate predominantly in SF, (*ii*) release the oxygen free radicals and proteolytic enzymes that destroy connective tissue components, and (*iii*) support chronic inflammation by secretion of numerous inflammatory mediators including, TNF-α and IL-1β (Edwards and Hallet, 1997; Babior, 2000). However, activated neutrophils are also the major source of Tau-Cl (Weiss et al., 1982), the compound exerting some anti-inflammatory activities (Marcinkiewicz, 1997). We reported that *in vitro* Tau-Cl inhibits several pathogenic functions of RA fibroblast-like synoviocytes (Kontny et al., 1999). Thus, it is conceivable that disturbed metabolism of Tau/Tau-Cl may contribute to RA pathogenesis. The aim of present study was to estimate the generation of Tau-Cl by neutrophils isolated from PB of healthy volunteers, as well as from PB and SF of RA patients.

Materials and methods

Chemicals and reagents

Gradisol G and phosphate buffered saline (PBS) were obtained from Polfa, Poland. All other chemicals were purchased from Sigma (St. Louis, MO).

Patients and cell isolation

All patients included in the study (n = 40; 30 females and 10 males; the mean age ± SD = 60.2 ± 13.4; the mean disease duration ± SD = 13.5 ± 10) fulfilled the American College of Rheumatology criteria for the diagnosis of rheumatoid arthritis (RA) (Arnett et al., 1988). Neutrophils were isolated from heparinised PB of adult healthy volunteers (n = 8; 4 females and 4 males) and RA patients (n = 28) by density gradient centrifugation with Gradisol G. Synovial fluids were obtained from knee puncture of 12 RA pa-

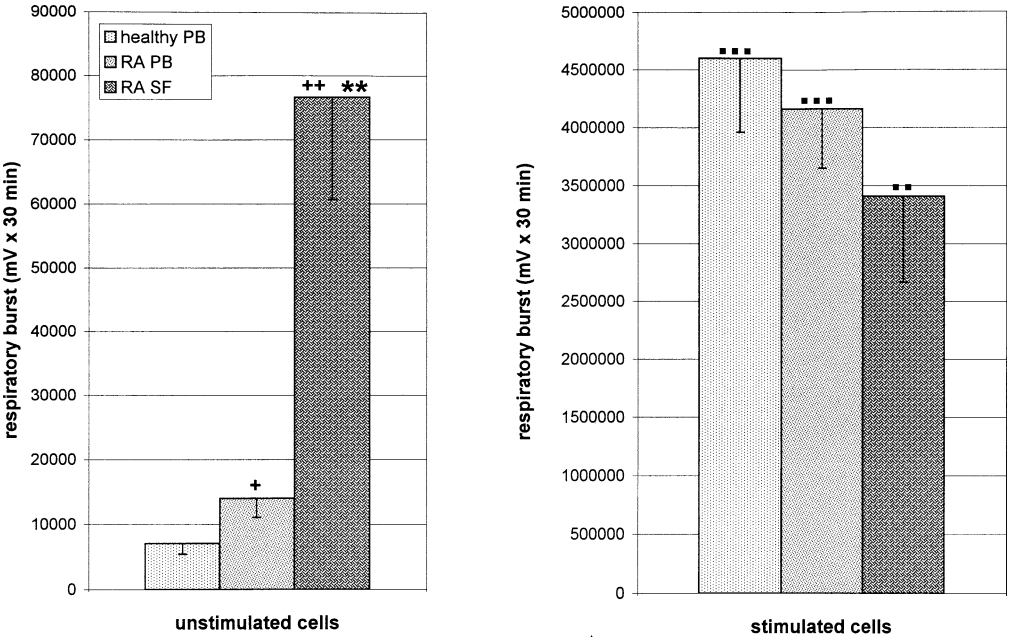

Fig. 1. Neutrophil respiratory burst. Neutrophils from PB of healthy volunteers (n = 8), RA PB (n = 28), or RA SF (n = 12) were stimulated with zymosan or left untreated (unstimulated cells). Reactive oxygen intermediate release was assayed as described in *Materials and methods*. Data are expressed as the mean ± SEM. $^{+}$ $0.05 > P > 0.01$, $^{++}$ $0.01 > P > 0.001$ (vs healthy PB); ** $0.01 > P > 0.001$ (RA SF vs RA PB); ■■ $0.01 > P > 0.001$, ■■■ $P < 0.0001$ (stimulated vs unstimulated cells)

tients, performed as a normal part of clinical care. Then neutrophils were isolated as described above. Cells were resuspended in the assay buffer (PBS supplemented with 0.1% bovine serum albumin and 0.1% glucose). The viability of cells, assayed by trypan blue exclusion, was ≥90% (SF) or ≥95% (PB).

Chemiluminescence assay

The assay was performed at 37°C in a final volume of 1 ml (1×10^6 cells), as described previously (Wojtecka-Łukasik et al., 1997). Zymosan particles were opsonised (for 30 min at 37°C) with human serum, washed with PBS and used (1 mg/ml) as a stimulus. Luminol (150 μM) and Tau (5–20 mM) were added prior to stimulus. The chemiluminescence response was recorded using BioOrbit 1251 luminometer, and total light generation after 30 min was analysed.

Statistical analysis

Results are expressed as the mean ± SEM. Statistical analysis was performed using the Student's *t*-test. $P < 0.05$ was considered significant.

Results and discussion

Spontaneous chemiluminescence of RA neutrophils, especially the cells isolated from SF, was markedly elevated (Fig. 1). It is consistent with observations that RA neutrophils exhibit several features indicative of partial activation (Edwards and Hallett, 1997). In accordance with previously described phenomenon of neutrophils "exhaustion" with respect to respiratory burst (Nurcombe et al., 1991), we also observed that RA SF neutrophils showed tendency to respond weaker than neutrophils of healthy volunteers to zymosan stimulation (Fig. 1).

Respiratory burst of activated neutrophils is accompanied by the formation of highly reactive oxidant hypochlorous acid (HOCl), produced by myeloperoxidase (MPO)-catalysed oxidation of Cl^- by H_2O_2. In neutrophil cytosol, a dominant free amino acid Tau is present at high (20 mM) concentration and acts as major scavenger for HOCl, which is stoichiometrically converted to relatively stable Tau-Cl (Babior, 2000). Taurine is often used as a trap for measurement of HOCl/Tau-Cl generation (Weiss et al., 1982; Cunningham et al., 1998). In the presence of physiologically relevant (15 mM) Tau concentration, the release of Tau-Cl (~100 nmol) by zymosan-stimulated neutrophils (2×10^6) is evident (Weiss et al., 1982). This is reflected by ≈50% inhibition of chemiluminescence response (Cunningham et al., 1998). Consistently, we report that zymosan-triggered chemiluminescence of PB neutrophils of both healthy volunteers and RA patients were inhibited with similar potency by Tau (Fig. 2). Importantly, the chemiluminescence response of RA SF neutrophils was inhibited less efficiently (Fig. 2), indicating that upon activation

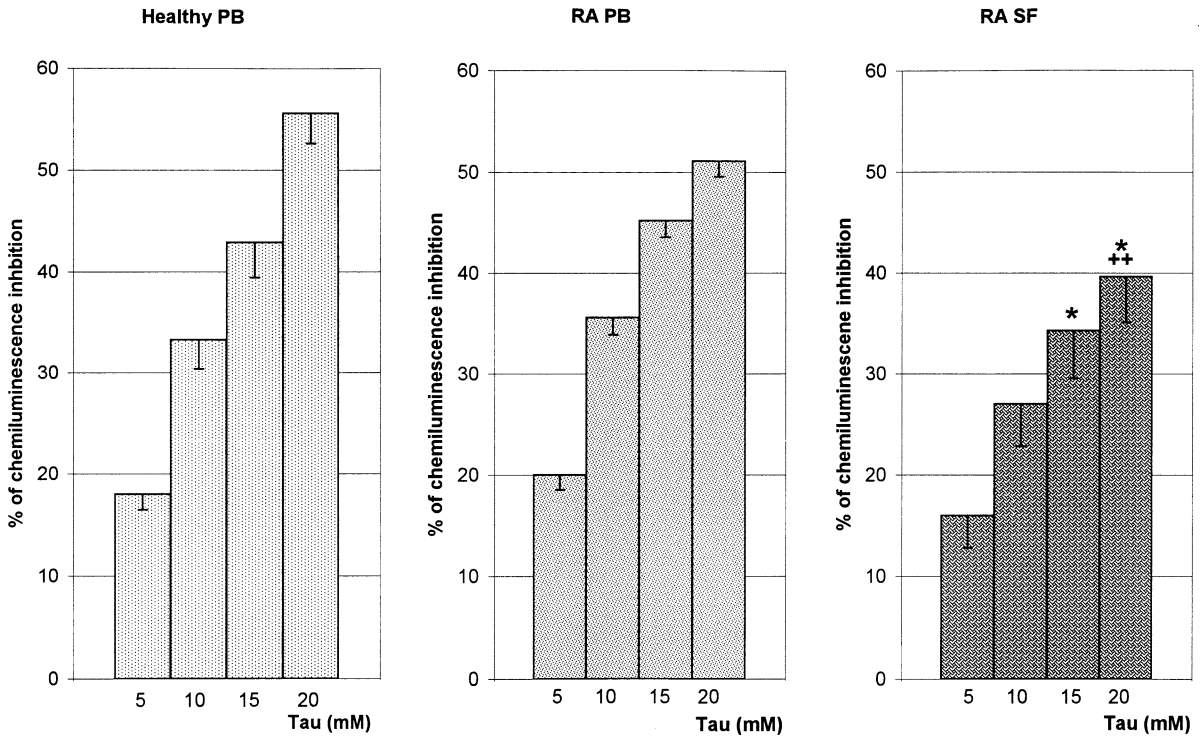

Fig. 2. Chemiluminescence response of neutrophils to zymosan in the presence of taurine (*Tau*). Neutrophils from healthy volunteers PB (n = 8), RA PB (n = 28), or RA SF (n = 12) were stimulated with zymosan in the presence or absence of Tau (control). Data represent the percentage of chemiluminescence inhibition in the presence of Tau compared to control, and are expressed as the mean ± SEM. In the presence of Tau (5–20 mM) the chemiluminescence responses of neutrophils were inhibited significantly ($0.01 > P$) in every group. $^{++}$ $0.01 > P > 0.001$ (vs healthy PB); * $0.05 > P > 0.01$ (RA SF vs RA PB)

these cells generate less Tau-Cl than neutrophils from peripheral blood. It is conceivable that diminished production of Tau-Cl by RA SF neutrophils is a consequence of reduced activities of NADPH-oxidase (Davies et al., 1990) and/or MPO (Nurcombe et al., 1991), noticed in these cells. *In vivo*, the presence of anti-neutrophil cytoplasmic autoantibodies found in 16–36% of RA patients that may affect neutrophil functions (Hoffman and Specks, 1998), as well as elevated plasma level of Tau (Trang et al., 1985), and hypertaurinuria (Rylance, 1969) suggesting abnormal Tau metabolism in these patients, may also be relevant.

Although Tau-Cl is weaker oxidant than HOCl, it is more stable and retains ability to oxidise similar targets as HOCl. Acting via this mechanism, Tau-Cl was reported to affect the activity of key enzymes implicated in the connective tissue homeostasis, resulting in inactivation of α_1-antiproteinase inhibitor (Carr et al., 2001) and activation of collagenase (Claesson et al., 1996). Despite the latter activity, generation of Tau-Cl was reported to protect collagen from HOCl-mediated degradation (Davies et al., 1993). Therefore, the main role of Tau-Cl seems to be the protection against HOCl-mediated tissue destruction. In addition, Tau-Cl exerts anti-inflammatory activities and may be engaged in the termination of inflammatory response (Marcinkiewicz, 1997). Consistently, we reported that Tau-Cl down-regulates pro-inflammatory cytokine (IL-6, IL-8) synthesis by RA fibroblast-like synoviocytes, and inhibits proliferation of these cells (Kontny et al., 1999). Moreover, we have also shown that Tau-Cl is a potent inhibitor of NFκB activity in these cells (Kontny et al., 2000). Importantly, NFκB plays pivotal role in the propagation of inflammatory response, because a broad range of genes encoding not only pro-inflammatory cytokines, but also growth factors, adhesion molecules, and stress proteins are activated by this transcription factor (May and Ghosh, 1998). Thus, Tau-Cl may play an important anti-inflammatory role and may also attenuate hyperplasia of synovial membrane in RA patients. Present results suggest impaired ability of RA SF neutrophils to generate Tau-Cl, and shed more light on the pathogenic

role of these cells in supporting chronic inflammation characteristic for this disease.

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