

Norepinephrine from Synovial Tyrosine Hydroxylase Positive Cells Is a Strong Indicator of Synovial Inflammation in Rheumatoid Arthritis

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ABSTRACT. Objective. Density of sympathetic nerve fibers in synovial tissue was lower in patients with rheumatoid arthritis (RA) compared to those with osteoarthritis (OA). This was accompanied by norepinephrine (NE) release from synovial tyrosine hydroxylase positive cells (TH+ cells). We investigated the role of TH+ cells and NE in synovial inflammation.

Methods. Synovial tissue of 34 patients with RA and 36 with OA who underwent knee joint replacement surgery was characterized using immunohistochemistry and a synovial tissue superfusion technique, respectively. In culture experiments with mixed synoviocytes, the effect of NE on secretion of interleukin 6 (IL-6), IL-8, tumor necrosis factor (TNF), and matrix metalloproteinase-3 (MMP-3) was investigated.

Results. Tissue density of TH+ cells was higher in RA compared to OA (63.9 vs 34.2 cells/mm²; $p = 0.017$). Basal NE release from synovial tissue correlated highly significantly with density of TH+ cells in RA ($R_{\text{rank}} = 0.573$, $p = 0.001$) but not in OA ($R_{\text{rank}} = 0.102$, NS). Basal NE release correlated with the degree of inflammation in RA ($R_{\text{rank}} = 0.420$, $p = 0.021$) but not in OA ($R_{\text{rank}} = 0.174$, NS), and with spontaneous IL-8 secretion in RA ($R_{\text{rank}} = 0.581$, $p = 0.001$) but not in OA ($R_{\text{rank}} = 0.160$, NS). Only in RA, density of TH+ cells correlated positively with spontaneous secretion of IL-6, IL-8, and MMP-3. We confirmed the extensive loss of sympathetic nerve fibers in RA compared to OA (0.32 vs 3.1 nerve fiber/mm²; $p < 0.001$). The ratio of sympathetic to sensory nerve fibers was 1 to 5 in RA and 2 to 1 in OA. A ratio of 1.0 separates almost all patients into 2 diseases groups (RA vs OA). Prior prednisolone treatment of RA patients was related to decreased spontaneous cytokine secretion, a lower density of T cells, CD163+ macrophages and TH+ cells, a lower degree of inflammation, and reduced synovial NE secretion. NE was able to inhibit secretion of IL-6 (in OA), IL-8 (in RA), and TNF (in RA and OA) in culture experiments.

Conclusion. TH+ cells and release of NE are strongly linked to a higher degree of synovial inflammation. Culture experiments indicate that NE has antiinflammatory properties at higher concentrations (10⁻⁵ M). NE secretion of TH+ cells may be an antiinflammatory mechanism to counteract local inflammation. Thus, TH+ cell derived NE can be an important local factor of immunomodulation in synovial inflammation. (J Rheumatol 2002;29:427-35)

Key Indexing Terms:

RHEUMATOID ARTHRITIS
NOREPINEPHRINE

SUBSTANCE P

CYTOKINE

SYMPATHETIC NERVES
MATRIX METALLOPROTEINASE 3

Nerve fibers and their neurotransmitters play an important role in the pathogenesis of synovial inflammation in rheumatoid arthritis (RA), as shown for proinflammatory sensory nerve fibers and substance P¹. The role within the sympathetic nervous system of the neurotransmitters norep-

inephrine (NE), neuropeptide Y (NPY), endogenous opioids, and ATP is not fully understood². For example, since NE binds to distinct G protein coupled receptor subtypes with functionally opposing downstream pathways ($\alpha_2 \rightarrow G\alpha_{i/o} \rightarrow$ low cyclic AMP; $\beta \rightarrow G\alpha_s \rightarrow$ high cyclic AMP), neuroimmunomodulation by NE depends on receptor affinity and the local concentration of the specific ligands. Thus, for example, proinflammatory effects of NE were attributed to α_2 -adrenergic receptor binding³. On the other side, NE binding to β -adrenoceptors was found to inhibit many immune functions such as secretion of tumor necrosis factor (TNF)^{4,5}, interferon- γ ⁶, interleukin 1 (IL-1)⁷, IL-12^{5,8}, natural killer cell activity *in vitro*^{9,10}, macrophage NO production¹¹, and many others. Treatment of collagen induced experimental arthritis with the β -adrenergic agonist salbutamol has shown a strong antiinflammatory effect. Thus, the local concentration of NE with its higher affinity

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Dr. Straub was supported by a grant from the Deutsche Forschungsgemeinschaft (DFG Str 511/5-1,2,3 and Str 511/11-1).

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Submitted June 5, 2001; revision accepted September 7, 2001.

for α -adrenoceptors is of outstanding importance for the immunomodulating effect of this neurotransmitter via either α or β -adrenoceptors.

We reported that tyrosine hydroxylase positive (TH+) sympathetic nerve fibers were drastically reduced in patients with RA compared to those with osteoarthritis (OA)¹². The ratio of the density of sympathetic to sensory nerve fibers was 1 to 5 in patients with RA and 1.5 to 1 in OA, which indicates a shift to a more proinflammatory situation in chronic RA compared to OA synovial tissue. That study also revealed spontaneous release of NE from synovial TH+ cells¹². However, the role of these NE secreting cells in the pathogenesis of synovial inflammation in RA is not understood. In this study, we aimed to confirm the loss of sympathetic nerve fibers in a significantly larger group of patients with RA and OA. We extended our research to the role of TH+ cells and NE in synovial inflammation in RA and OA. As well, we wished to characterize the immunomodulating properties of NE in early cultures of mixed synoviocytes using IL-6, IL-8, TNF, and matrix metalloproteinase-3 (MMP-3) as indicators of synovial inflammation.

MATERIALS AND METHODS

Patients. Thirty-four patients with RA and 36 with OA who underwent knee joint replacement surgery were consecutively included without further selection. Patients were informed about the purpose of the study and gave written consent. The study was approved by the Ethical Committee of the University of Regensburg. Clinical and laboratory data were recorded (Table 1). Variables such as erythrocyte sedimentation rate, C-reactive protein, blood leukocyte count, and rheumatoid factor (latex test) were measured by standard techniques. No patient had received prior intraarticular injection of corticosteroids.

Synovial tissue preparation and isolation of synovial cells. Synovial tissue samples were obtained immediately after opening the knee joint capsule. The preparation of the tissue for histology was as described^{12,13}. Briefly,

Table 1. Basic characteristics of patients with OA and RA who underwent knee joint replacement surgery. Data are given as means \pm SEM; percentages are given in parentheses and ranges in brackets.

	OA	RA
No. of patients	36	34
Age, yrs	70.2 \pm 1.2 [41–82]	64.4 \pm 1.3 [39–80]
Duration of disease, yrs	9.8 \pm 1.7	9.1 \pm 1.7
ESR, mm	15.3 \pm 1.6*	31.7 \pm 3.3
CRP, mg/l	17.6 \pm 3.8*	34.2 \pm 4.8
Blood leukocyte count, nl	7.1 \pm 0.4*	8.6 \pm 0.4
Rheumatoid factor	NA	24 (70.6)
Medication		
Prednisolone	NA	26 (76.5)
Daily prednisolone mg	NA	4.3 \pm 0.5 [0–20]
Methotrexate	NA	7 (20.6)
Sulfasalazine	NA	2 (5.9)
Azathioprine	NA	2 (5.9)
Oral or injectable gold	NA	1 (2.9)
NSAID	17 (47.2)	26 (76.5)

* $p < 0.005$ vs patients with RA; NA: not applicable. All patients with methotrexate were also treated with prednisolone.

one piece of synovial tissue about 9 cm² was dissected. Fat tissue and tissue with a large number of vessels were removed. Twenty-four pieces of about 16 mm² were loaded into 24 superfusion chambers, and 8 pieces roughly 1 cm² of the same synovial area were used for histology. Samples intended for hematoxylin–eosin (H&E) and alkaline phosphatase-antialkaline phosphatase (APAAP) staining were immediately placed in protective freezing medium (Tissue-Tek, Sakura Finetek Europe, Zoeterwoude, The Netherlands) and then quick-frozen floating on liquid nitrogen. Tissue samples for the detection of nerve fibers were fixed for 12 to 24 h in phosphate buffered saline (PBS) containing 4% formaldehyde and then incubated in PBS with 20% sucrose for 12 to 24 h. The tissue was then bedded in Tissue-Tek and quick-frozen. Each patient's synovial tissue samples were stored at -80°C .

Synovial cells were isolated by enzymatic digestion of fresh synovial tissue for 1 h at 37°C (Dispase Grade II, Boehringer, Mannheim, Germany). The cells were seeded in Dulbecco's modified Eagle's medium (DMEM; Biochrom, Berlin, Germany) and cultured 12 h (early culture of mixed synoviocytes). The percentage of different types of synoviocytes was tested by specific antibodies against prollyl 4-hydroxylase (for the synoviocyte type B = fibroblasts; clone 3-2B12, Calbiochem, Bad Soden, Germany) and CD68 (synoviocyte type A = macrophages; clone PG-M1, Dako, Hamburg, Germany)¹⁴. Twelve hours after enzymatic digestion, about 10% expressed the CD68 macrophage marker and 20% could be stained positively for the fibroblast enzyme prollyl 4-hydroxylase, reflecting a mixed population of synoviocytes (synoviocyte type A to B = 1 to 2; the rest of the cells may have lost these surface markers after 12 h). Twenty thousand cultured synovial cells were used for stimulatory tests with NE, as described below.

Histological evaluation of inflammation and determination of synovial innervation. Histological evaluation was as described¹². Briefly, the frozen tissue samples were cut into 6–8 μm thick sections and cell density and lining layer thickness were evaluated using standard H&E staining of about 45 sections. At 400 \times magnification, the extent of the lining layer thickness was determined by averaging the number of cells in a lining layer cross section at 9 different locations. The cell density in the synovial tissue was determined by counting all stained cells in 17 randomly selected high power fields (400 \times) and expressed per mm². To determine the number of T cells (CD3, Dako), macrophages (CD163, Dako) and capillary vessels (collagen IV, Dako) in the synovial tissue of each patient, 8 cryosections were investigated using APAAP staining and the number of identified structures was averaged from 17 randomly selected high power fields (400 \times) and expressed per mm².

Determination of synovial innervation has been described¹². Briefly, 6 to 8 cryosections 7–9 μm thick were used for immunohistochemistry with primary antibodies against tyrosine hydroxylase (TH, the key enzyme for NE production in sympathetic nerve endings; Chemicon, Temecula, CA, USA) and substance P (SP, the key transmitter of the sensory nerve fibers; Chemicon). A Cy3 labeled secondary antibody (Dianova, Hamburg, Germany) was used to achieve an immunofluorescent staining. The numbers of stained nerve fibers and TH+ cells per mm² were determined by averaging the number of stained nerve fibers with a minimum length of 50 μm (determined by a small micrometer eyepiece) or TH+ cells in 17 randomly selected high power fields (400 \times).

Inflammation index. The morphological and functional inflammatory variables were combined to establish a mathematical index of an individual patient (IXp) in relation to the disease group:

The subscript av indicates the average value of all patients in a disease group (RA or OA, respectively), and subscript p indicates that this is the individual value of a patient (RA or OA). CD means overall cell density, IL-6 means spontaneous IL-6 superfusate concentration at 2 h, IL-8 means spontaneous IL-8 superfusate concentration at 2 h, LL means lining layer thickness, MD means macrophage density, TD means T cell density, and VD means vascularity. This technique allows the combination of all variables even with very different numerical values and units. The inflammation index IXp gives an indication whether a patient has more or less

inflammation in relation to the other patients of the same disease group ($IXp > 100$ = more inflammation, $IXp \leq 100$ = less inflammation compared to the mean value of the entire group). An overall inflammation index for both disease groups was calculated in a similar way using the average value of all patients (RA and OA). The overall inflammation index gives an idea whether a patient has more or less inflammation in relation to all investigated patients.

Cytokine analysis in the superfusate and in culture supernatants. In the superfusion experiments (see superfusion technique, below), cytokine concentrations were determined in a superfusate fraction of roughly 1 ml (collected over 15 min). Human IL-6, IL-8 (detection limit in the 2 assays < 2 pg/ml; Endogen, Boston, MA, USA), and MMP-3 (detection limit 3 ng/ml, Amersham, Braunschweig, Germany) were determined by enzyme immunometric assay. Since in superfusion experiments TNF was not measurable in about 50% of patients, we did not include TNF as an indicator.

In culture experiments with isolated cells, supernatant was collected 16 h after the start of NE incubation and stored in adequate aliquots at -80°C for determination of cytokines. The enzyme immunometric assays for human IL-6, IL-8, and MMP-3 were used. Moreover, human TNF (detection limit 0.15 pg/ml; HS Quantikine, R&D, Wiesbaden, Germany) was also determined by enzyme immunometric assay. Intraassay and interassay coefficients of variation for all ELISA were $< 10\%$.

Superfusion technique of synovial tissue and measurement of NE. As described for spleen slices¹⁵⁻¹⁷, we used a microsperfusion chamber apparatus to superfuse pieces of synovial tissue with culture medium (RPMI-1640, 25 mM HEPES, 5% fetal calf serum, 1% penicillin/streptomycin, 30 μM mercaptoethanol, 0.57 mM ascorbic acid, 1.3 mM calcium; all Sigma, Deisenhofen, Germany). These superfusion chambers had a volume of 80 μl . Superfusion was performed for 2 h at a temperature of 37°C and a flow rate of 66 $\mu\text{l}/\text{min}$ (one piece per chamber, 24 chambers in parallel). Superfusate was collected at 60 min and 120 min to measure spontaneous NE and cytokine release (1 ml). The superfusate was immediately frozen and stored for short time periods at -20°C (maximum 3 days). Cytokines were measured in the 120 min fraction as described (1 ml). The amount of NE in the superfusate was measured at 60 and 120 min by radioimmuno-metric assay (RIA; IBL, Hamburg, Germany). The highly sensitive protocol used with this kit provides a detection limit of 10 pg/ml. Test samples analyzed with HPLC showed that the RIA produced comparable results. The NE values obtained at 60 and 120 min were averaged and expressed in pg/ml (spontaneous NE secretion).

Modulation of cytokine secretion by NE. We used the main neurotransmitter of the sympathetic nervous system in relevant concentrations: 10^{-5} to 10^{-8} M (Artereno[®], Höchst, Frankfurt, Germany). NE was prepared immediately before use. Twenty thousand mixed synovial cells were stimulated for 16 h in 1 ml DMEM plus 100 μl medium with the adequate neurotransmitter concentration to obtain the noted final concentrations. The cells were not stimulated further by additional substances because they spontaneously produced large amounts of the tested cytokines IL-6, IL-8, TNF, and MMP-3. Due to the different tissue digestion time of RA tissue (needs more time) compared to OA tissue (needs less time), we believe that absolute values of cytokine supernatant concentrations should not be used for inter-disease comparison of the cytokine secreting capability of RA or OA synovial cells.

Statistical analysis. All data are given as mean \pm SEM. Correlations were calculated by Spearman rank correlation analysis (SPSS/PC, Advanced Statistics, v 10.0.1, SPSS Inc., Chicago, IL, USA) and graphically illustrated by linear regression analysis. Group means were compared by the nonparametric Mann-Whitney test (SPSS). $p < 0.05$ was the significance level.

RESULTS

Histological inflammation in patients with RA and OA. The

trend toward a difference in the inflammatory measures between the 2 disease groups seen in our earlier study now reached significant levels in almost all variables due to the larger number of subjects: lining layer thickness (RA vs OA: 11.3 ± 2.2 vs 3.8 ± 0.6 cells; $p < 0.001$), CD3+ T cell density (155.2 ± 25.3 vs 74.3 ± 11.0 cells/ mm^2 ; $p = 0.008$), CD163+ macrophage density (439.4 ± 62.3 vs 313.8 ± 53.1 cells/ mm^2 ; $p = 0.042$), TH+ cell density (63.9 ± 10.8 vs 34.2 ± 5.82 cells/ mm^2 ; $p = 0.017$), and overall cellularity (1113.5 ± 62.7 vs 737.0 ± 41.7 cells/ mm^2 ; $p < 0.001$) were significantly higher in patients with RA compared to OA. However, there was no significant difference in vascularity (166.2 ± 15.3 vs 146 ± 9.4 vessels/ mm^2 ; NS). As expected, this indicates that histological inflammation is higher in RA than OA.

Synovial innervation and spontaneous NE release. We found very low numbers of TH+ sympathetic nerve fibers in RA compared to OA patients (0.32 ± 0.1 vs 3.1 ± 0.4 nerve fibers/ mm^2 ; $p < 0.0001$). In contrast, density of SP+ sensory nerve fibers was elevated in RA compared to OA patients (2.5 ± 0.2 vs 1.5 ± 0.2 nerve fibers/ mm^2 ; $p = 0.001$). The ratio of the density of TH+ nerve fibers/SP+ nerve fibers was markedly lower in RA compared to OA patients [0.22 ± 0.05 vs 2.15 ± 0.18 (dimensionless); $p < 0.0001$].

In receiver-operator characteristic analysis, we tried to identify a cutoff value for the ratio of TH+ nerve fibers/SP+ nerve fibers that can separate 2 groups with a low or high overall inflammation index ($IXp \leq 100$, $IXp > 100$; 100 = mean of all patients, see Materials and Methods). The ROC analysis revealed a cutoff value for the nerve fiber ratio of 1.00 with an optimum specificity of 60.5%, optimum sensitivity of 66.7%, and optimum accuracy of 63.1%. As shown in Figure 1A, separation of all patients into 2 groups according to the nerve fiber ratio shows that patients with a ratio ≤ 1.00 (31 with RA, 2 with OA; Figure 1A) had significantly more histologically proven inflammation compared to patients with a ratio > 1.00 (1 with RA, 31 with OA; Figure 1A). This indicates that a nerve fiber ratio ≥ 1.00 is associated with a lower degree of synovial inflammation, and this cutoff significantly separates the 2 disease groups with a sensitivity of 94.2% and a specificity of 97.1% (Figure 1B).

Interestingly, spontaneous NE secretion from synovial pieces in the superfusion apparatus was similar in RA compared to OA patients (147.8 ± 24.8 vs 185.4 ± 34.6 pg/ml; NS). As observed in an earlier study, there exists a strong indication for another source of NE in RA synovium, which was found to be a TH+ cell¹². This is corroborated by a strong correlation between spontaneous NE secretion and density of TH+ cells in RA but not in OA patients, where sympathetic nerves are still present (Figure 2).

Synovial inflammation and spontaneous NE secretion or density of TH+ cells. To determine the role of NE producing TH+ cells in inflammation, the following analyses were

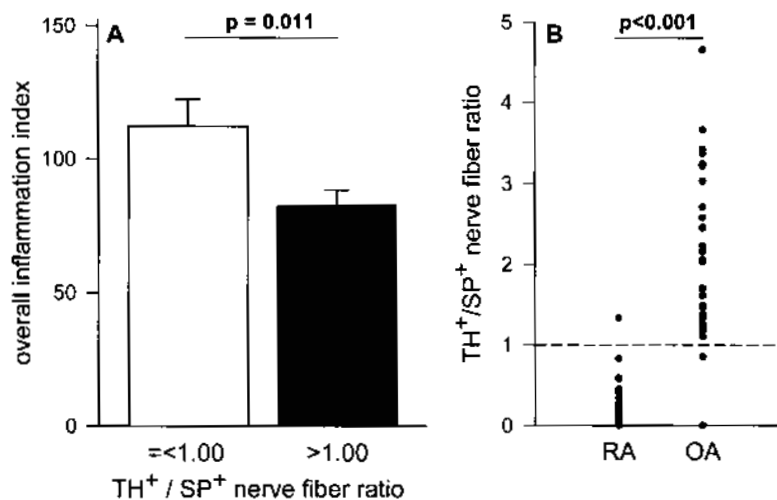


Figure 1. Nerve fiber ratio and inflammation in RA and OA patients. A. Comparison of the average overall inflammation index of the 2 groups with a nerve fiber ratio ≤ 1.00 and > 1.00 . B. Nerve fiber ratio in patients with RA and OA. The nerve fiber ratio is a continuous variable calculated by dividing the density of tyrosine hydroxylase positive (TH+) nerve fibers by the density of substance P positive (SP+) nerve fibers (dimensionless).

carried out. It is obvious that there is a correlation between the disease-specific inflammation index and spontaneous NE secretion in RA but not in OA (Figure 3).

In a correlation matrix analysis, we found that the density of TH+ cells correlated with spontaneous release of IL-6, IL-8, MMP-3, and NE in RA but not OA (Table 2). This indicates that TH+ cells may be involved in secretion of

these mediators. Table 2 further reveals that spontaneous NE secretion was only related to density of TH+ cells but not to the density of other identified structures. Together with our earlier findings¹², the latter finding corroborates that TH+ cells are the producers of NE. Vascularity did not correlate with the concentration of soluble immune mediators.

Since IL-8 is a proinflammatory mediator due to its

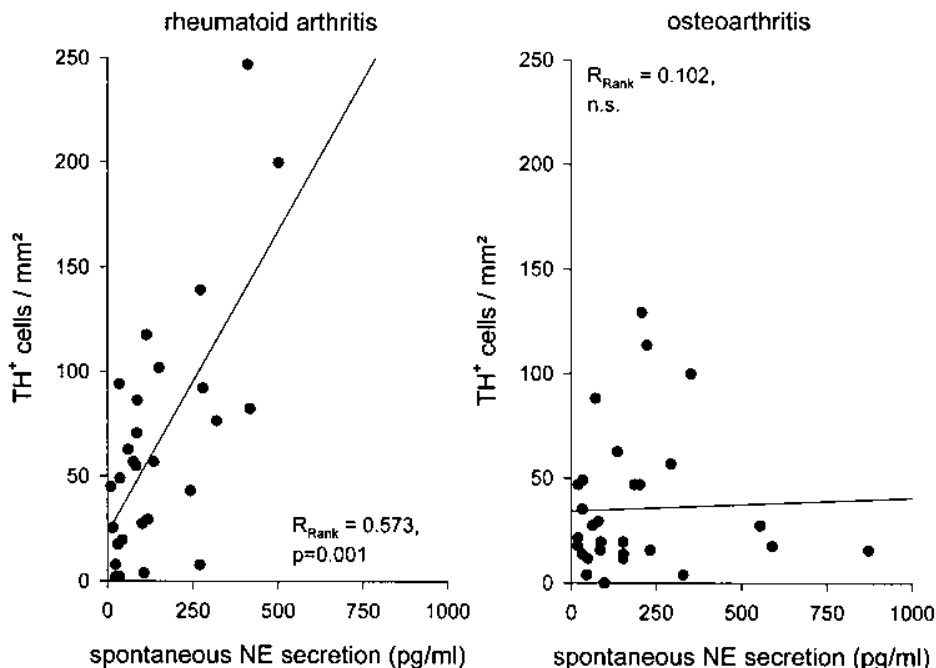


Figure 2. Correlation between spontaneous norepinephrine (NE) secretion and density of tyrosine hydroxylase positive (TH+) cells in patients with RA and OA. The linear regression line, Spearman rank correlation coefficient R_{Rank} , and p value are given in the respective panel.

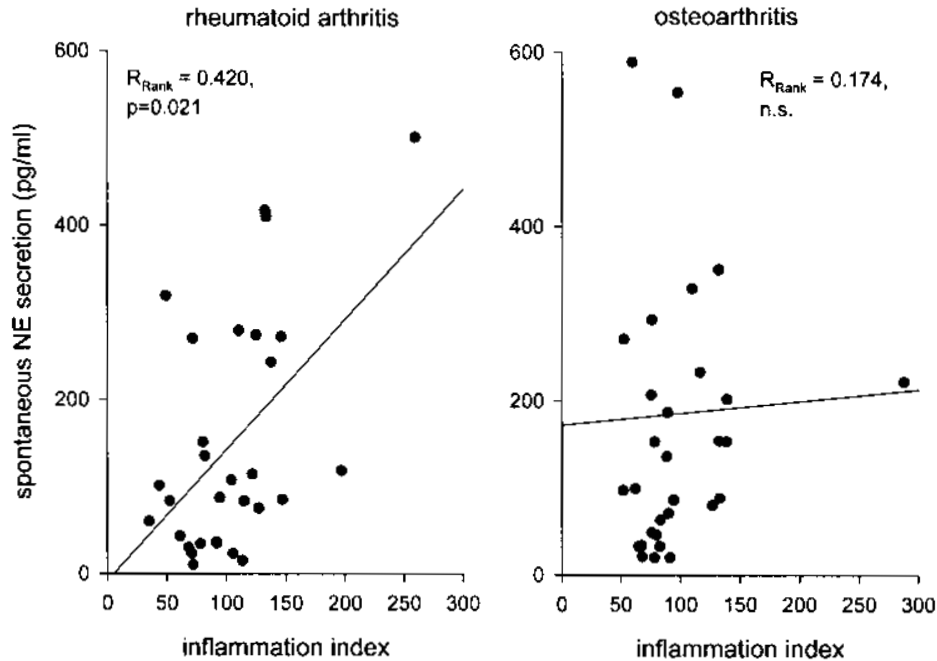


Figure 3. Correlation between disease-specific inflammation index and spontaneous norepinephrine (NE) secretion in patients with RA and OA. The linear regression line, Spearman rank correlation coefficient R_{Rank} , and p value are given in the respective panel.

capacity to attract proinflammatory neutrophils to the joint, we investigated the interrelation of the spontaneous secretion of IL-8 and NE as well as the spontaneous secretion of IL-8 and density of TH+ cells (Figure 4). It can be seen that there is a strong positive correlation between spontaneous IL-8 and these other variables in RA, but not in OA.

Influence of NE on secretion of IL-6, IL-8, TNF, and MMP-3 from synoviocytes. To extend the studies, we investigated the influence of NE on the spontaneous secretion of IL-6, IL-8, TNF, and MMP-3 from mixed synoviocytes (Table 3). NE tended to inhibit all cytokines, which only reached the significance level for IL-6 in OA, IL-8 in RA,

TNF in RA and OA (at higher concentrations), and MMP-3 in OA. In all cases, NE inhibited but did not stimulate secretion of these mediators. This is particularly true for the proinflammatory cytokine TNF (Table 3).

Influence of prior therapy for RA on spontaneous secretion of local mediators and histological measures of inflammation. Prior prednisolone therapy in patients with RA significantly reduced the spontaneous secretion of IL-8 and MMP-3 measured in the superfusate (Figure 5A). Further, patients with prior prednisolone treatment presented a decreased density of T cells, CD163+ macrophages, and TH+ cells (Figure 5B). Patients with prior prednisolone

Table 2. Matrix of Spearman correlation coefficients for the relation between various secreted mediators and other indicators of inflammation in patients with RA and OA. Coefficients for OA are given in parentheses.

	Spontaneous IL-6	Spontaneous IL-8	Spontaneous MMP-3	Spontaneous NE
Cellularity	NS (NS)	NS (-0.467) [†]	NS (NS)	NS (NS)
T cells	0.390* (NS)	0.540 [†] (NS)	0.613* (NS)	NS (NS)
CD163+ macrophages	0.368* (NS)	0.391* (NS)	0.391* (NS)	NS (NS)
TH+ cells	0.440* (NS)	0.401* (NS)	0.562* (NS)	0.573 [‡] (NS)
Lining layer	NS (NS)	NS (NS)	NS (NS)	NS (NS)

* p < 0.05, [†] p < 0.006, [‡] p = 0.001. MMP-3: matrix metalloproteinase 3 (n = 15 cases); NE: norepinephrine; NS: not significant.

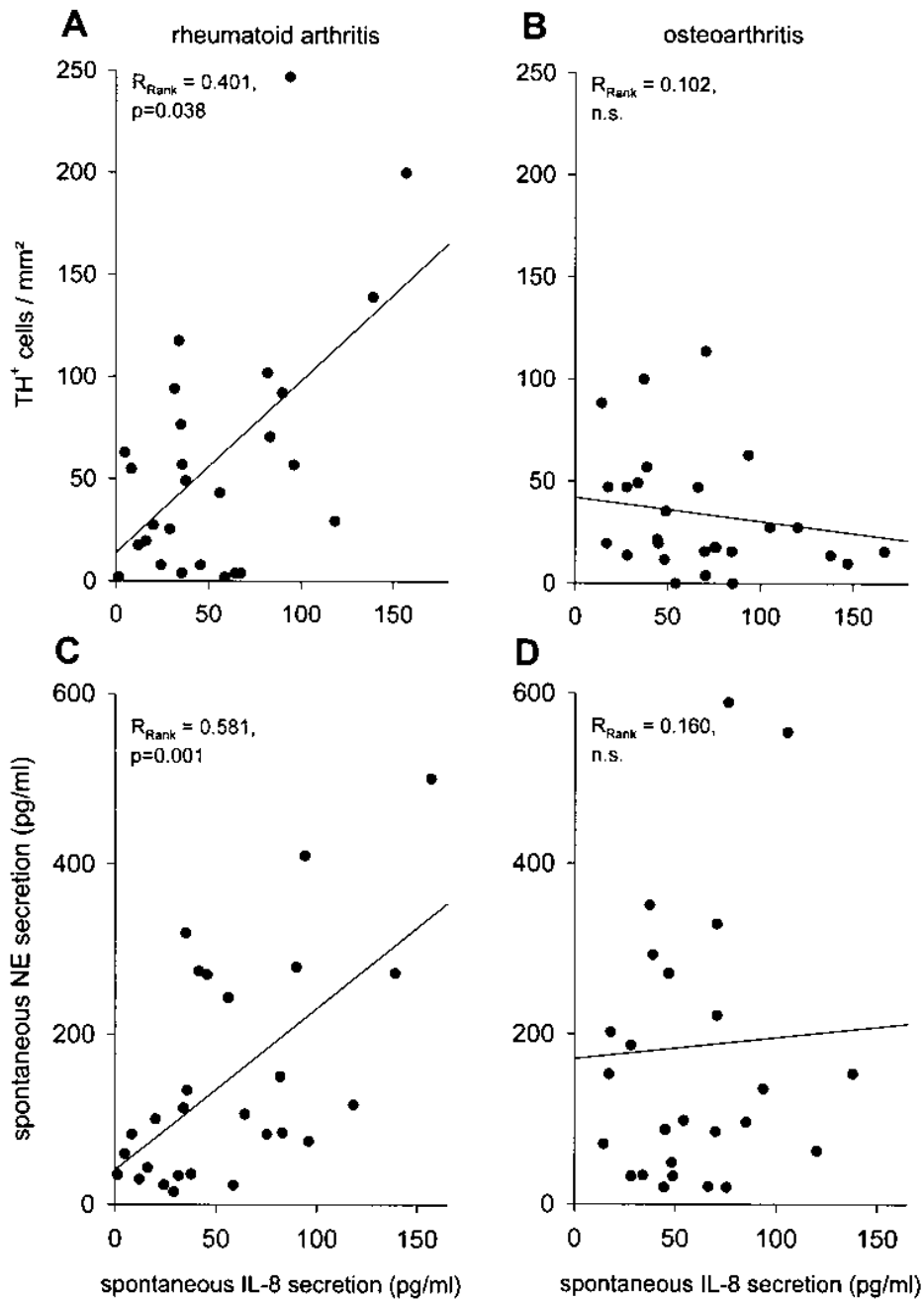


Figure 4. Correlation between spontaneous IL-8 secretion and density of tyrosine hydroxylase positive (TH+) cells (A, B) or spontaneous norepinephrine (NE) secretion (C, D) in patients with RA and OA. The linear regression line, Spearman rank correlation coefficient R_{rank} , and p value are given in the respective panel.

therapy had a lower inflammation index (Figure 5C) and tended to have reduced spontaneous NE secretion (Figure 5D). Patients with prior combined therapy of methotrexate and prednisolone compared to patients without combined treatment presented a lower vascularity (with vs without therapy: 112 ± 17 vs 172 ± 16 vessels/mm²; $p = 0.029$) and tended to have lower density of CD163+ macrophages (307

± 71 vs 523 ± 83 macrophages/mm²; $p = 0.084$).

DISCUSSION

This study revealed that patients with RA in comparison to patients with OA had increased histological inflammation, extensively reduced density of sympathetic nerve fibers, increased density of sensory nerve fibers, and increased density of TH+ cells. Spontaneous NE secretion and density

Table 3. Influence of norepinephrine on spontaneous secretion of IL-6, IL-8, TNF, and MMP-3 in patients with RA (n = 3) and OA (n ≥ 4). Each concentration was tested in 4 separate wells for each patient. Data are given as means ± SEM.

	IL-6	IL-8	TNF	MMP-3
Rheumatoid arthritis				
10 ⁻⁵ M	85.7 ± 14.9	75.2 ± 13.4*	62.8 ± 9.3*	79.0 ± 10.2
10 ⁻⁶ M	82.2 ± 13.4	78.8 ± 14.1*	70.8 ± 9.6*	84.0 ± 10.7
10 ⁻⁷ M	78.7 ± 13.8	73.9 ± 12.9*	67.2 ± 8.1*	74.1 ± 8.8
10 ⁻⁸ M	75.0 ± 13.3	75.0 ± 13.9*	82.7 ± 9.3	77.5 ± 9.4
Control	100.0 ± 18.3	100.0 ± 15.2	100.0 ± 12.6	100.0 ± 12.5
Osteoarthritis				
10 ⁻⁵ M	79.2 ± 7.0*	89.8 ± 7.4	64.7 ± 2.3 [†]	79.9 ± 2.8 [†]
10 ⁻⁶ M	82.7 ± 6.6*	94.3 ± 7.0	70.7 ± 2.9 [†]	79.5 ± 3.1 [†]
10 ⁻⁷ M	79.6 ± 6.6*	89.2 ± 7.5	86.5 ± 2.8	73.7 ± 1.8 [†]
10 ⁻⁸ M	71.6 ± 4.8**	91.0 ± 6.8	89.8 ± 1.9	74.2 ± 1.4 [†]
Control	100.0 ± 8.6	100.0 ± 5.8	100.0 ± 4.5	100.0 ± 2.4

*p < 0.05, **p < 0.01, [†]p < 0.001 in comparison to the respective control (OA: mean control for IL-6 = 2819, for IL-8 = 1955, for TNF = 18.6 pg/ml, and for MMP-3 = 65.2 ng/ml; RA: mean control for IL-6 = 1854, for IL-8 = 1125, for TNF = 24.4 pg/ml, and for MMP-3 = 10.2 ng/ml).

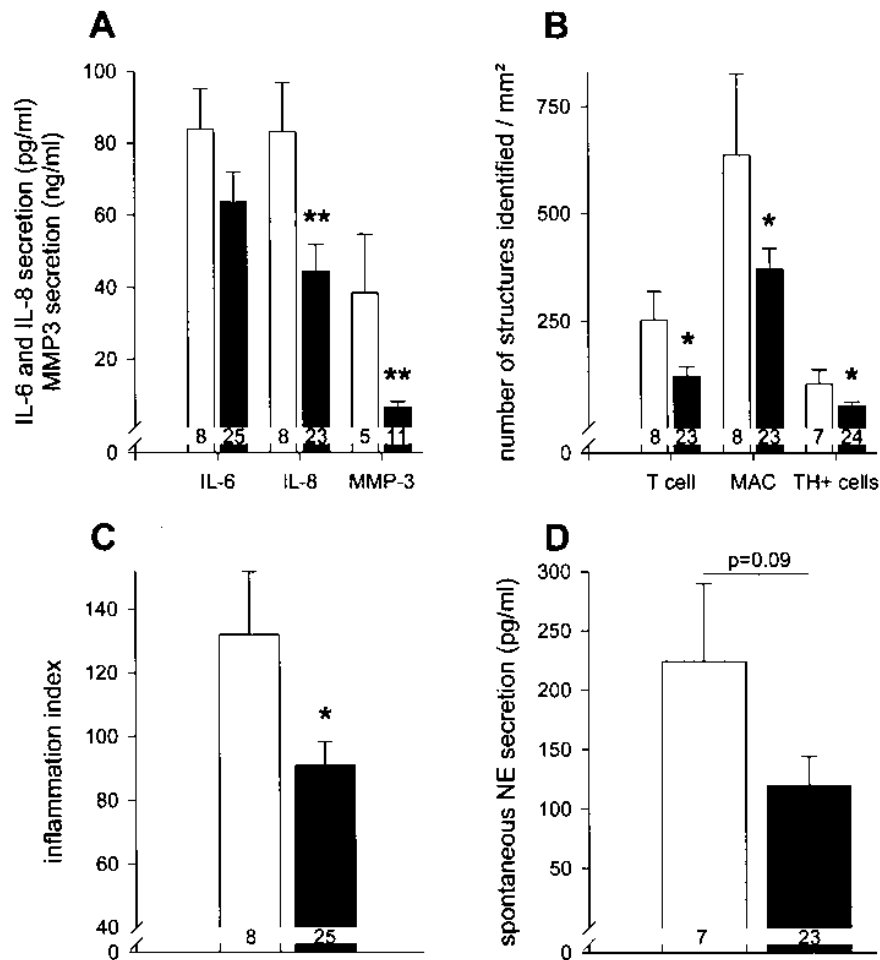


Figure 5. Influence of prior prednisolone therapy in patients with RA on histological and functional inflammation variables. White/black bars indicate RA patients without/with prior prednisolone therapy. Influence on (A) spontaneous secretion of IL-6, IL-8, and matrix metalloproteinase-3 (MMP-3); (B) density of histologically identified structures (MAC: CD163+ macrophage, TH+: tyrosine hydroxylase positive cells); (C) disease-specific inflammation index; (D) spontaneous secretion of norepinephrine. The numbers of patients with complete data are given in the bars. *p < 0.05, **p < 0.01 for difference vs the respective group without prior prednisolone therapy.

of TH+ cells strongly correlated with histological and functional measures of inflammation and NE inhibited cytokine secretion *in vitro*. Prior prednisolone therapy is associated with reduced histological inflammation and cytokine secretion.

This study fully supports our earlier investigation in a smaller number of patients with RA in which we described drastically reduced density of TH+ sympathetic nerve fibers in synovial tissue¹². A new finding is that the ratio of sympathetic to sensory nerve fibers was 1 to 5 in RA patients and 2 to 1 in OA. A ratio of 1.0 separates almost all patients into 2 disease groups (RA/OA) with a high sensitivity and specificity and into 2 groups with a different degree of synovial inflammation: patients with a ratio ≤ 1.0 had a significantly higher degree of synovial inflammation compared to patients with a ratio > 1.0 . Since SP, an important neurotransmitter of sensory nerve fibers, was found to be proinflammatory in many instances^{1,20-25}, a preponderance of SP+ nerve fibers in relation to sympathetic nerve fibers may be a significant pathogenic factor in RA. Since sympathetic neurotransmitters such as NE and adenosine are only antiinflammatory at high concentrations as ligands of the β -adrenoceptor and the A2 adenosine receptor^{4,26-31}, retraction of sympathetic nerve fibers or functional alterations may maintain a proinflammatory situation in the synovial tissue. It is interesting that a ratio of sympathetic nerve fibers to sensory nerve fibers of 1 to 1 indicates a critical ratio where sympathetic and sensory nerves are in a balance. A preponderance of sympathetic nerve fibers indicates a lower inflammatory situation and a preponderance of sensory nerve fibers indicates an increased inflammatory situation.

TH+ cells can take over NE production instead of sympathetic nerve fibers, and spontaneous NE release becomes similar in patients with RA and OA¹². Now we have confirmed these earlier findings in a larger group of patients. Spontaneous NE secretion was found to correlate with the density of TH+ cells in RA but not in OA, which suggests that TH+ cells play an important role in NE production only in patients with RA. Production of hormones and neurotransmitters by immune cells seems to be a general feature when these cells are stimulated: immune cells can produce, for example, adrenocorticotrophic hormone³², 17 β -estradiol³³, histamine³⁴, norepinephrine⁴, opioids³⁵, acetylcholine³⁶, and many others. In the synovial tissue of patients with RA, large amounts of NE are produced by TH+ cells in the absence of TH+ sympathetic nerve fibers. This study clearly indicates that the density of these TH+ cells and the amount of NE secretion are linked to a more proinflammatory situation in patients with RA, but not in patients with OA, who still have a significant number of TH+ sympathetic nerve fibers in the tissue. It may be that NE production by these cells is a feature of their proinflammatory activation in the synovial microenvironment. The question remains

whether or not NE of TH+ cells is proinflammatory or anti-inflammatory.

In additional studies, we have now demonstrated that NE is particularly able to inhibit secretion of TNF and IL-8 of mixed synoviocytes in patients with RA. Since both cytokines are typical representatives of proinflammatory mediators, it is thought that local NE production may be necessary to counterbalance proinflammatory pathways. However, inhibition of TNF secretion is only possible at high concentrations of 10^{-5} and 10^{-6} M NE, which may exist under conditions with an increased nerve firing rate and high nerve fiber density^{18,19}. We do not exactly know the NE concentration in the superfused synovial tissue because the NE concentration in the superfusate of 100 pg/ml (roughly 0.6×10^{-9} M) does not reflect the true concentration in the tissue due to fast degradation of the molecule and reuptake into the nerve ending. Thus, the question remains whether NE secretion by synovial cells leads to a high enough local concentration of this neurotransmitter to dampen TNF or IL-8 secretion in the synovial tissue.

This study further confirms that prior administration of prednisolone reduces histological and functional signs of inflammation. The trend for a reduction of NE secretion by prednisolone may be an unspecific antiinflammatory effect of this hormone leading to decreased density of TH+ cells and probably a lower activation state of these cells. Somewhat similar is the effect of prior concomitant therapy with methotrexate plus prednisolone, which leads to reduced synovial vascularity.

The reduction of sympathetic nerve fibers and the increase of sensory SP+ nerve fibers in RA is accompanied by production of norepinephrine most probably from TH+ cells. Since the systemic antiinflammatory feedback systems — the hypothalamic-pituitary-adrenal axis and the hypothalamus-autonomic nervous system axis — are necessary to dampen proinflammatory pathways in the periphery, it may be very important that the major mediators cortisol and NE exert their effects in a time related and dose related fashion. Uncoupling of the 2 major antiinflammatory systems with a lack of adequate cortisol secretion and reduction of sympathetic nerve fibers may support continuous smoldering inflammation in the synovial tissue.

ACKNOWLEDGMENT

We thank Angelika Gräber for excellent technical assistance.

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