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

LUITPOLD E. MILLER, JOACHIM GRIFKA, JÜRGEN SCHÖLMERICH, and RAINER H. STRAUB

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 (Rrank = 0.573, p = 0.001) but not in OA (Rrank = 0.102, NS). Basal NE release correlated with the degree of inflammation in RA (Rrank = 0.420, p = 0.021) but not in OA (Rrank = 0.174, NS), and with spontaneous IL-8 secretion in RA (Rrank = 0.581, p = 0.001) but not in OA (Rrank = 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 CYTOKINE SYMPATHETIC NERVES NOREPINEPHRINE SUBSTANCE P 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 P. The role within the sympathetic nervous system of the neurotransmitters norepinephrine (NE), neuropeptide Y (NPY), endogenous opioids, and ATP is not fully understood. For example, since NE binds to distinct G protein coupled receptor subtypes with functionally opposing downstream pathways (α2 → Gαi/o → low cyclic AMP; β → GαS → 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 α2-adrenergic receptor binding. 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-γ6, interleukin 1 (IL-1)7, IL-128, natural killer cell activity in vitro9,10, 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...
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. 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 described. 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× 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×) 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×) and expressed per µm².

Determination of synovial innervation has been described. 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×).

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 synovial inflammation.

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

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

* p < 0.005 vs patients with RA; NA: not applicable. All patients with methotrexate were also treated with prednisolone.
inflammation in relation to the other patients of the same disease group (IXp > 100 = more inflammation, IXp ≤ 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. Intrassay 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 microsuperfusion 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 µl/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 radioimmunoassay (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: NE 10−3 to 10−6 M (Arterenol®, 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 interdisease comparison of the cytokine secreting capability of RA or OA synovial cells.

Statistical analysis. All data are given as mean ± 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/mm²; p = 0.008), CD163+ macrophage density (439.4 ± 62.3 vs 313.8 ± 53.1 cells/mm²; p = 0.042), TH+ cell density (63.9 ± 10.8 vs 34.2 ± 5.82 cells/mm²; p = 0.017), and overall cellularity (1135.3 ± 62.7 vs 737.0 ± 41.7 cells/mm²; 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/mm²; 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/mm²; 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/mm²; 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 ≤ 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...
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 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 (dimension-
less).

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 coeffi-
cient $R_{\text{max}}$, 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.

<table>
<thead>
<tr>
<th></th>
<th>Spontaneous IL-6</th>
<th>Spontaneous IL-8</th>
<th>Spontaneous MMP-3</th>
<th>Spontaneous NE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellularity</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>(NS)</td>
<td>(NS)</td>
<td>(NS)</td>
<td>(NS)</td>
<td>(NS)</td>
</tr>
<tr>
<td>T cells</td>
<td>0.390*</td>
<td>0.540*</td>
<td>0.613*</td>
<td>NS</td>
</tr>
<tr>
<td>(NS)</td>
<td>(NS)</td>
<td>(NS)</td>
<td>(NS)</td>
<td>(NS)</td>
</tr>
<tr>
<td>CD163+ macrophages</td>
<td>0.368*</td>
<td>0.391*</td>
<td>0.391*</td>
<td>NS</td>
</tr>
<tr>
<td>(NS)</td>
<td>(NS)</td>
<td>(NS)</td>
<td>(NS)</td>
<td>(NS)</td>
</tr>
<tr>
<td>TH+ cells</td>
<td>0.440*</td>
<td>0.401*</td>
<td>0.562*</td>
<td>0.573*</td>
</tr>
<tr>
<td>(NS)</td>
<td>(NS)</td>
<td>(NS)</td>
<td>(NS)</td>
<td>(NS)</td>
</tr>
<tr>
<td>Lining layer</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>(NS)</td>
<td>(NS)</td>
<td>(NS)</td>
<td>(NS)</td>
<td>(NS)</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>IL-6</th>
<th>IL-8</th>
<th>TNF</th>
<th>MMP-3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rheumatoid arthritis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10^-3 M</td>
<td>85.7 ± 14.9</td>
<td>75.2 ± 13.4*</td>
<td>62.8 ± 9.3*</td>
<td>79.0 ± 10.2</td>
</tr>
<tr>
<td>10^-4 M</td>
<td>82.2 ± 13.4</td>
<td>78.8 ± 14.1*</td>
<td>70.8 ± 9.6*</td>
<td>84.0 ± 10.7</td>
</tr>
<tr>
<td>10^-5 M</td>
<td>78.7 ± 13.8</td>
<td>73.9 ± 12.9*</td>
<td>67.2 ± 8.1*</td>
<td>74.1 ± 8.8</td>
</tr>
<tr>
<td>10^-6 M</td>
<td>75.0 ± 13.3</td>
<td>75.0 ± 13.9*</td>
<td>82.7 ± 9.3</td>
<td>77.5 ± 9.4</td>
</tr>
<tr>
<td>Control</td>
<td>100.0 ± 18.3</td>
<td>100.0 ± 15.2</td>
<td>100.0 ± 12.6</td>
<td>100.0 ± 12.5</td>
</tr>
<tr>
<td><strong>Osteoarthritis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10^-3 M</td>
<td>79.2 ± 7.0*</td>
<td>89.8 ± 7.4</td>
<td>64.7 ± 2.3</td>
<td>79.9 ± 2.8†</td>
</tr>
<tr>
<td>10^-4 M</td>
<td>82.7 ± 6.6*</td>
<td>94.3 ± 7.0</td>
<td>70.7 ± 2.9</td>
<td>79.5 ± 3.1†</td>
</tr>
<tr>
<td>10^-5 M</td>
<td>79.6 ± 6.6*</td>
<td>89.2 ± 7.5</td>
<td>86.5 ± 2.8</td>
<td>73.7 ± 1.8†</td>
</tr>
<tr>
<td>10^-6 M</td>
<td>71.6 ± 4.8**</td>
<td>91.0 ± 6.8</td>
<td>89.8 ± 1.9</td>
<td>74.2 ± 1.4†</td>
</tr>
<tr>
<td>Control</td>
<td>100.0 ± 8.6</td>
<td>100.0 ± 5.8</td>
<td>100.0 ± 4.5</td>
<td>100.0 ± 2.4</td>
</tr>
</tbody>
</table>

*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 tissue. 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, 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, 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 OA. 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, adrenocorticotropic hormone, 17β-estradiol, histamine, norepinephrine, opioids, acetylcholine, 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 antiinflammatory.

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. 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.

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REFERENCES
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