INVolvement of Neurotrophins and Their Receptors in Spontlyoarthritis Synovitis: Relation to Inflammation and Response to Therapy

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ABSTRACT

Objective: To investigate whether the expression of the four members of the neurotrophin family and their four corresponding receptors is related to synovial inflammation in spondyloarthritis (SpA) patients.

Material and Methods: SF and serum neurotrophins and their receptors were measured by ELISA. Immunohistochemistry was used for synovial tissue (ST) biopsies from patients with SpA, rheumatoid arthritis (RA), and osteoarthritis (OA). In SpA synovium, immunoreactivity of the receptors trkA and NGFRp75 was also assessed before and after 12 weeks of treatment with the monoclonal anti-TNF-alpha antibody infliximab.

Results: mRNA transcripts of all neurotrophins and receptors were expressed in the inflamed synovium. At the protein level, BDNF and NT-3 were significantly higher in SF of SpA as compared to OA. In contrast, ELISA of serum samples showed that the highest member in SpA was NT-4. Immunohistochemistry demonstrated that the neurotrophin receptors trkA and NGFRp75 were highly expressed in the inflamed synovium of SpA patients, correlating with vascularity and lymphoid aggregates, respectively. In addition, immunoreactivity of both receptors was significantly decreased after infliximab treatment.

Conclusions: Neurotrophins and their receptors are expressed in inflamed peripheral joints of SpA patients. The findings indicate that their expression is not constitutive but related to inflammation and that they might be involved in the local disease processes.

Key Words: spondyloarthritis, neurotrophins, trkA, histopathology, synovial tissue
INTRODUCTION
We have previously validated cDNA-based microarrays as screening approach to identify and further explore potential pathogenic mediators of synovitis in spondyloarthritis (1, 2). Recent data suggest the upregulation of neurotrophins (NTs) and their receptors in SpA (3). NTs are secreted growth factors exerting essential functions such as proliferation, survival and differentiation of cells within the mammalian nervous system upon binding to and dimerizing of specific tyrosine kinase receptors (4). Up to date, nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and NT-4 have been identified in humans. Their specific high-affinity receptors are trkA (for NGF), trkB (for BDNF and NT-4), as well as trkC (for NT-3). All neurotrophins also bind to a common low-affinity receptor, NGFRp75, signaling independently from the trk receptors (4).

Over the last decade, substantial evidence has demonstrated expression of NTs in non-neural tissues (4). One major source for NTs and their receptors outside the nervous system appears to be cells of the hematopoietic/immune system as well as the skeletal/connective tissue system (5-11). Indeed, expression of NGF and its high-affinity receptor (trkA) has been described in arthritic synovium and chondrocytes (5, 12, 13). While this may be related to neural alterations such as the reduction of sympathetic innervation of inflamed synovium (5, 14, 15), an increasing body of evidence suggests a functional role of neurotrophins and their receptors in immune-mediated inflammatory diseases (16, 17).

In this context, the present study aimed to determine the expression of neurotrophins and their receptors in peripheral synovitis of SpA patients and to explore their potential involvement in the inflammatory disease processes.

MATERIAL AND METHODS

Patients
Clinical data of all 49 patients are given in Table 1. The study population consisted of 24 SpA patients fulfilling the ESSG classification criteria (18), 15 RA patients fulfilling the ACR classification criteria (19) being included as inflammatory comparison, and 10 OA patients as non-inflamatory comparison. Nine of the 24 SpA patients were treated with the monoclonal anti-TNF-alpha antibody infliximab (5 mg/kg IV at baseline, week 0, and week 6) as described previously (20). In these patients synovial tissue samples were obtained at baseline as well as at week 12 of therapy. Written informed consent was obtained prior to study entrance as approved by the local Ethics Committee.

Synovial tissue and fluid samples
In all patients, ST biopsies and SF samples were obtained by needle arthroscopy of an actively inflamed knee joint as described earlier (21). Since we previously demonstrated that the global histopathological features of the synovium are identical in the various SpA subgroups (22, 23), we considered the SpA samples (8 ankylosing spondylitis, 6 psoriatic arthritis, and 10 undifferentiated SpA) collectively as one group. In the patients receiving anti-TNFalpha treatment, synovial biopsies were obtained at baseline and after 12 weeks of treatment. Histological inflammation was assessed by two independent observers, scoring semi-quantitatively the lining layer thickness, vascularity, global inflammatory infiltration, lymphoid aggregates, polymorphonuclear cells, plasma cells, CD3+ T lymphocytes, and CD20+ B lymphocytes as extensively described before (2, 20, 24). Data are depicted in Table 1.

Real time-PCR (Taqman assay)
Using TRizol® reagent (Life Technologies, Eggenstein/Germany) total RNA was extracted from synovial biopsy specimens of four patients with SpA (n=2) and RA (n=2). Generation of cDNA by reverse transcription and usage of Taqman assay followed the manufacturer’s protocol. Assays-on-demand for NGF-beta (assay-ID Hs00171458), BDNF (Hs00156058), NT-3 (Hs00267375), and an
assay-by-design for NT-4 (cat. no. 4332078) as well as assays-on-demand for the receptors trkA (Hs00176787), trkB (Hs00178811), trkC (Hs00176797), and NGFRp75 (Hs00609976) were purchased from Applied Biosystems (Warrington, UK).

Quantitative Immunoassay (ELISA)
Quantitative sandwich enzyme immunoassay kits were purchased from R&D Systems (Abingdon, UK). Concentrations of the neurotrophic factors NGF (cat. no. DY256), BDNF (DBD00), NT-3 (DY267), and NT-4 (DY268) were measured in the synovial fluid samples of 15 SpA, 15 RA, and 10 OA patients described in Table 1. For comparison, paired serum samples of the patients as well as serum from 10 healthy subjects were used as controls.

Immunohistochemistry
Immunohistochemistry was performed on frozen ST sections in order to determine the immunoreactivity in the synovial lining layer, the sublining layer, and the endothelium of the high-affinity receptor trkA (anti-trkA rabbit polyclonal antibody, clone sc-7268, Santa Cruz/CA) and the low-affinity receptor NGFRp75 (anti-NGFRp75 monoclonal mouse antibody, clone M3507, DAKO, Glostrup/Denmark) in 24 SpA, 15 RA, and 10 OA patients. Immunohistochemical staining was performed using the LSAB+ kit (Dako) and was scored by two independent observers in a blinded fashion on a scale from 0 (no staining intensity) to 3 (maximum staining intensity). This scoring method was extensively described and validated previously (2, 20, 24).

In order to determine a potential modulation of the two neurotrophin receptors by the monoclonal anti-TNF antibody infliximab, we stained for trkA and NGFRp75 in ST biopsies obtained prior to and 12 weeks after treatment in 9 of the 24 SpA patients (Table 1). Stained sections were blinded for patients (not pair-wise) and time of sampling and scored by two independent observers.

Statistical analysis
Between group comparison was performed with the unpaired or paired (for the pre- and post-infliximab samples) non-parametric Mann Whitney U test as appropriate. Correlation coefficients within one group were determined using Spearman Rho correlation matrices as appropriate for non-parametric data. The level of significance was defined as p<0.05.

RESULTS
Neurotrophin and neurotrophin receptor mRNA is expressed in the inflamed synovium
All four neurotrophins and all four receptors were found to be expressed in inflammatory synovium samples obtained from 2 SpA and 2 RA patients (data not shown). Highest levels were measured for NGF and NT-3 (up to 8500fold) and for trkB, trkC, and NGFRp75 (up to 8800fold) in both SpA and RA as compared to four healthy PBMC. However, mRNA of NT-4 and trkA were expressed on lower levels (up to 15fold). While this analysis was not aimed to perform a quantitative comparison between SpA and RA, it confirmed the presence of neurotrophin mRNA in the inflamed synovium of both the diseases.

SF levels of BDNF and NT-3 are elevated in SpA
In order to investigate at the protein level whether neurotrophins were present in the inflamed joint, we measured their concentration in SF samples by ELISA. Individual values are depicted by four scatter plots for each neurotrophin in Figure 1 (panels A-D). Detectable concentrations of BDNF were measured in the majority of SpA patients revealing significantly higher concentrations as compared to OA patients (p=0.025). In addition, NT-3 was significantly higher in SpA when compared to OA (p=0.047). The remaining neurotrophins were detectable only in the minority of samples.

In order to investigate whether NTs were expressed only locally or also found in the serum, we analysed their concentration in the serum of all patients using paired SF and serum samples and also in 10 samples of healthy controls. Individual values are depicted again by four scatter plots (Figure
NT-4 was expressed in the majority of all samples revealing significantly higher concentrations in all disease groups as compared to healthy controls. Surprisingly, BDNF was detected in the serum of all 50 individuals revealing highest levels in healthy controls which were significantly higher than in all other groups (Figure 1E). None of the samples expressed relevant amounts of NGF or NT-3. Both for SF and serum levels, there were no differences between the SpA subgroups.

Correlations of neurotrophins between serum and SF samples as determined by Spearman Rho test were significant in SpA for NGF (r=0.73, p=0.002) and NT-3 (r=0.65, p=0.008) but in none of the other groups. Taken together, these data indicate that both BDNF and NT-3 levels are elevated in SpA SF, with NT-3 being found predominantly locally in the inflamed joint whereas BDNF is also elevated in the serum.

**trkA and NGFRp75 are highly expressed in SpA synovitis**

Using immunohistochemistry, we next analyzed the synovial expression of the high-affinity receptor trkA and the low-affinity receptor NGFRp75 in 24 SpA, 15 RA, and 10 OA patients. As shown in Figure 2 (panels A-C) and Figure 3 (panels A-F), the staining for trkA was most pronounced in the lining and the sublining layer and less intensive in the endothelium. NGFRp75 staining was predominantly found in the sublining layer and to a lower extent in the endothelium of SpA patients (Figure 2, panels D-F; Figure 3, panels G-L). Comparing the different disease groups, trkA immunoreactivity in the lining layer was significantly higher in SpA when compared to RA and OA patients (p=0.047 for SpA vs RA and p=0.034 for SpA vs OA). The fact that there was no difference for lining layer hyperplasia between these groups emphasizes the specificity of this finding (Table 1). Moreover, trkA in the sublining layer was significantly higher in SpA vs OA as well as in RA vs OA (p=0.001 and p=0.003, respectively). Besides, NGFRp75 in the endothelium was significantly higher in SpA when compared to RA and OA (p=0.012 and p=0.041, respectively), which may be related to the higher degree of global vascularity in SpA (Table 1). There were no differences in trkA or NGFRp75 expression between SpA subgroups.

**Correlation of neurotrophins and their receptors with local inflammation in SpA synovitis**

Since both neurotrophins (BDNF and NT-3) and neurotrophin receptors (trkA and NGFRp75) appeared to be highly expressed in SpA synovitis, we then analyzed whether their expression in SpA patients (n=15 for ELISA data, n=24 for immunohistochemistry data) correlated with parameters of synovial inflammation. Interestingly, SF NT-3 levels correlated inversely with the degree of vascularisation (r=-0.73; p=0.003). As to the receptors, significant correlations were found for trkA in the lining layer and the degree of vascularisation (r=0.53; p=0.009) as well as NGFRp75 in the endothelium and the number of lymphoid aggregates (r=0.63; p=0.002), (Figure 4).

**trkA and NGFRp75 immunoreactivity is downregulated by anti-TNF-alpha treatment**

Since the high expression in SpA synovitis and the correlations with parameters of local inflammation suggested that the synovial NT receptor expression was related to local inflammation, we analyzed whether trkA and NGFRp75 expression could be modulated by TNF-alpha blockade in vivo. The immunohistochemistry procedure used here is identical as described for data depicted in figure 2 and 3. As illustrated in Figure 5, trkA immunoreactivity was most intense in the lining layer at baseline and was found to be significantly reduced after 12 weeks of treatment (median [range]: 2 [1-3] versus 1 [1-3]; p=0.03), whereas staining in the sublining layer and the endothelium was not significantly altered. For NGFRp75, immunoreactivity was significantly reduced in the sublining layer (2 [0-3] versus 1 [0-1]; p=0.004) as well as in the endothelium (2 [0-3] versus 0 [0-2]; p=0.004), (Figure 6).

**DISCUSSION**

We have previously identified neurotrophins and their receptors as being expressed in SpA synovitis using cDNA based microarrays (3). Since recent studies suggest that this system may also play a role...
in immunity, inflammation and infection (25, 26, 27), and since we recently demonstrated that candidate mediators identified by microarray may turn out to be important factors in SpA inflammation (2), the global aim of the present study was to provide detailed evidence that neurotrophins and their receptors are expressed in SpA synovitis.

Although the aim of the PCR experiments was to confirm the expression at the mRNA level in ST rather than doing quantitative comparisons, it appeared to be that NT-3, BDNF, trkB, and trkC were highly expressed whereas NT-4 and trkA were expressed on lower levels. Paralleling these findings, quantitative immunoassay indicated the presence of BDNF and NT-3 but not NT-4 and NGF in SF. While these data do not exclude that the different factors are expressed in the synovial tissue itself rather than in SF, they indicate the local presence of BDNF and NT-3 proteins in the inflamed SpA joint. Both neurotrophins were higher and more frequently detected in SF of SpA than in OA.

Of interest, BDNF levels were higher in serum than in SF, with the highest concentrations being found in the serum of healthy subjects. While this is in agreement with previous reports on serum BDNF levels (28, 29, 30), this could suggest that BDNF is diffusing from serum rather than being produced locally. However, this hypothesis does not fit with the presence of mRNA in ST, with the higher SF levels in an inflammatory disease such as SpA versus OA, with the absence of correlation between BDNF levels in serum and SF, and with the fact that NT-4 is found in serum but obviously does not diffuse to the SF in the same individuals. For NT-3, the situation is more clear-cut since it was detected more frequently and at higher levels in SF than in serum, suggesting local production. The presence of at least BDNF and NT-3 in the inflamed SpA joint raises the possibility that they exert a local effect through interaction with their receptors. Both PCR and immunohistochemistry confirmed the extensive presence of the receptors in the inflamed SpA joint, which in turn extends previous studies demonstrating up-regulation of trkA expression in experimental arthritis models (12), in human osteoarthritic chondrocytes (7), and in the human rheumatoid synovium (5). Our stainings revealed a different pattern for the high-affinity NGF receptor trkA, which was extensively found in the lining layer as well as in the sublining layer, and the low-affinity NGFRp75 receptor, which was found predominantly in the sublining layer and the endothelium. Several studies indicate a generally lower expression of NGFRp75 as compared to the high-affinity receptor trkA (12, 32, 33). Interestingly, the immunoreactivity in the synovial membrane was clearly distinct for the two receptors. Indeed, the elevated expression of both neurotrophins and their receptors in chronic autoimmune arthritis and especially in SpA and also the distinct expression pattern of the two receptors raise the possibility for a biological role in SpA synovitis. A functional role for trkA, the high-affinity NGF receptor, has not been demonstrated in arthritis so far but has been recently demonstrated in psoriasis: a pharmacological blocking agent as well as NGF-neutralizing antibodies clearly inhibited the disease in a mouse model (33). In addition, NGF and trkA seem to play a pathogenic role in allergic bronchial asthma (16). Consistent with these data in other immune-mediated inflammatory diseases, we found evidence in the present study for a correlation between the expression of the neurotrophin receptors and histological parameters of inflammation such as vascularity and the presence of lymphoid aggregates in SpA synovium. The fact that the expression of these receptors is not constitutive but is actively modulated in relation to inflammation in vivo was further demonstrated by the significant reduction of immunoreactivity of the two receptors by an effective pharmacological therapy with infliximab. Taken together, these data indicate that reduction of synovial inflammation by anti-TNF-alpha treatment is paralleled by a profound downregulation of the neurotrophin receptor expression.

It is interesting to note that in a previous study, NGF and trkA were found to be activated by LPS incubation in healthy human monocytes/macrophages (34). When neutralizing anti-NGF antibody was added, massive apoptosis of the cells was noted suggesting NGF as an autocrine survival factor for monocytes/macrophages in infection and inflammation. Similar observations were reported in virally infected monocytes, mast cells, and memory B lymphocytes (25, 26, 35). Considering the pronounced role of macrophages in SpA inflammation and their reduction by anti-TNF-alpha therapy, this might be one of the mechanisms linking neurotrophins to synovial inflammation (36, 37, 38, 39). However, their role is likely to be much more complex, including anti-inflammatory and protective aspects as suggested for NGF and NT-3 in experimental colitis (40, 41). Another function
attributed to neurotrophins may also include tissue repair/remodelling capacities. NGF in particular has been attributed to play a role in wound healing, fibrosis, and treatment of vascular as well as corneal ulcers (35, 42, 43, 44).

In conclusion, the present study demonstrates the presence of both neurotrophins and their receptors in peripheral SpA synovitis. We also provide in vivo evidence that the expression of these mediators is not constitutive and might be directly or indirectly related to the inflammatory disease process. Neurotrophins and their receptors might function as potential immunomodulatory mediators and warrant further investigations in SpA.

**AFFILIATIONS**
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REFERENCES


38. Demetter P, De Vos M, Van Huysse JA, Baeten D, Ferdinand L, Peeters H, Mielants H, Veys EM, De Keyser F, Cuvelier CA. Colon mucosa of both spondyloarthritis and Crohn's...


### Table 1

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### Legend Table 1

Demographic and clinical data from the 49 patients included in the study: 24 spondyloarthritis (SpA), 15 rheumatoid arthritis (RA), and 10 osteoarthritis (OA) patients. All patients had active disease with at least one swollen knee joint. Among the 24 SpA patients, 9 were studied also after 12 weeks of treatment with the monoclonal anti-TNF-alpha antibody infliximab. Data are given as median (range). dd, disease duration; SJC, swollen joint count; CRP, C-reactive protein serum levels; ESR, erythrocyte sedimentation rate.

The following synovial histology parameters were evaluated: lining, microscopical evaluation of the lining layer thickness ranging from 1 (layer of 1-2 cells) to 2 (layer of 4-5 cells) to 3 (layer of >5 cells); vasc, degree of vascularisation reaching from 0-3; infiltr, degree of cellular infiltration reaching from 0-3; LA, presence of lymphoid aggregates reaching from 0 (absent) to 1 (present); PC, presence of plasma cells reaching from 0 (absent) to 3 (multiple cells within the entire synovium); PMN, presence of polymorphonuclear cells within the synovium reaching from 0 (absent) to 3 (multiple cells within the entire synovium); immunoreactivities of CD3+, T cell marker (anti-CD3 monoclonal antibody, DAKO) and CD20+, B cell marker (anti-CD20 monoclonal antibody, DAKO) were graded from 0-3.

### Figure 1

*see separate file*
Legend Figure 1
Neurotrophins were measured by quantitative immunoassay in synovial fluids (SF) of 15 spondyloarthritis (SpA), 15 rheumatoid arthritis (RA), and 10 osteoarthritis (OA) patients (panels A-D) as well as in the serum of these patients including 10 healthy controls (HC), (panels E-H). The scatter plots of all four neurotrophins indicate the individual values. Bold horizontal lines represent the median. Detectable concentrations (median, range; always given for the total group of patients) of SF BDNF were measured in 11/15 (73%) SpA patients (4.5 pg/ml, 0-90), in 8/15 (53%) RA patients (1.7 pg/ml, 0-218), and in 2/10 (25%) OA patients (0 pg/ml, 0-24). SF BDNF concentrations were significantly higher in the SpA group as compared to OA patients (panel A * p=0.025). SF NT-3 was detected in 5/15 (33%) SpA patients (0 pg/ml, 0-200), and in 4/15 (27%) RA patients (0 pg/ml, 0-17). SF NT-3 levels were also significantly higher in SpA when compared to OA (panel C * p=0.047). Serum BDNF (panel E) was detectable in all 50 individuals revealing significantly higher levels in HC as compared to all other groups (SpA * p<0.0001, RA * p<0.0001, and OA * p=0.001), whereas serum NT-4 (panel H) in SpA (* p<0.001), RA (* p=0.001), and OA (* p=0.008) was significantly higher as compared to HC.

Figure 2
see separate file
Legend Figure 2
Immunoreactivity of trkA (panels A-C) and NGFRp75 (D-F) in the lining layer, sublining layer, and endothelium of synovial tissue biopsies obtained from clinically involved knee joints in spondyloarthritis (SpA, n=24), rheumatoid arthritis (RA, n=15), and osteoarthritis (OA, n=10). The results represent the individual scores on a semi-quantitative 0 (no expression) to 3 (high expression) scale, with bars representing the median score. As indicated (*), the immunoreactivity of trkA was significantly higher in SpA lining than in RA lining (p=0.047) and OA lining (p=0.034). Similarly, trkA was higher expressed in SpA and RA sublining compared to OA (p=0.001 and p=0.003, respectively). NGFRp75 was almost absent in the lining layer and was equally expressed across disease groups in the sublining layer. However, some SpA patients showed high endothelial expression, resulting in a significant difference compared to RA (p=0.012) and OA (p=0.041).

Figure 3 A-F, G-L
see 2 separate files
Legend Figure 3 A-L
Microscopic pictures of synovial tissue sections from 12 different patients are depicted. Figure 3, images A-F show trkA immunostaining of synovial tissue samples of 2 different SpA, RA, and OA patients each at low (A, B, C, x160) and high magnification (D, E, F, x320). In SpA patients, intensive trkA staining predominantly in the lining layer (A) and less intense staining in the sublining layer and the endothelium (D) is observed. In RA patients, a similar pattern for trkA staining is observed (B, x160 and E, x320) and correspondently in the right column for OA patients. NGFRp75 immunoreactivity is shown in images G-L revealing an intense staining in the sublining layer (G, J) of SpA patients. However, immunoreactivity for NGFRp75 is less intense in RA (H, K) and OA patients (I, L) as compared with SpA patients. In all 3 groups, NGFRp75 staining is not observed in the lining layer, however, it is found also in the endothelium of SpA patients.

Figure 4
see separate file
Legend Figure 4
Significant correlations as determined by Spearman Rho test in SpA patients between neurotrophin expression and parameters of synovial inflammation were significant for: NT-3 in SF and degree of vascularisation (A), immunoreactivity of trkA in the lining layer and degree of vascularisation (B) as
well as immunoreactivity of NGFRp75 in the endothelium and the number of lymphoid aggregates (C).

**Figure 5**
*see separate file*

**Legend Figure 5**

Immunohistochemistry on ST biopsies of selected SpA patients before and after 12 weeks of infliximab treatment (original magnification is x160 in A, B and x320 in C, D). Images on the left (A, C) show trkA staining at baseline (week 0) with a predominance of immunoreactivity in the lining and sublining layer. Staining of synovial samples obtained in the same patients after 12 weeks of treatment with infliximab are shown in B and D (week 12). The three pair plots (E-G) on the right side indicate the semiquantitative scores (0-3) for trkA expression in the lining and sublining layer as well as in the endothelium of all 9 spondyloarthritis (SpA) patients which were analysed before and 12 weeks after TNFalpha-blocking treatment with infliximab. Panel E: the trkA reduction in the lining layer is found significant at p=0.03 (*).

**Figure 6**
*see separate file*

**Legend Figure 6**

Immunohistochemistry on ST biopsies of selected SpA patients before and after 12 weeks of infliximab treatment (original magnification is x160 in A, B and x320 in C, D). Images A and C reveal NGFRp75 staining at baseline with a predominance in the sublining layer and the endothelium. B and D show synovial sections of the same patients stained after 12 weeks of anti-TNF treatment with infliximab, revealing a clearly less intense or even absent staining in both the lining and the sublining layer as well as in the endothelium. Again, three pair plots (E-G) on the right side indicate the semiquantitative scores (0-3) of NGFRp75 expression in the lining and sublining layer as well as in the endothelium of the 9 SpA patients before and 12 weeks after TNFalpha-blocking treatment with infliximab. Panels F and G: NGFRp75 is significantly reduced in the sublining layer as well as in the endothelium (both at * p=0.004).
A: $r = -0.73$, $p = 0.003$

B: $r = 0.53$, $p = 0.009$

C: $r = 0.63$, $p = 0.002$