Letters to the Editor

Nerve growth factor in the synovia of patients with rheumatoid arthritis: Correlation with TNF-α and IL-1β and possible functional significance

Sir,

Nerve growth factor (NGF) is a neurotrophin that plays a crucial role in the growth, differentiation and function of neurons of the peripheral and central nervous system and is implicated in the regulation of immune and inflammatory responses, through direct or indirect action on immunocompetent cells (1).

We have previously reported that rheumatoid arthritis (RA), both in animal models and in humans, is characterized by elevated amounts of NGF in the synovial fluid (2), and that the knee joints of tumor necrosis factor (TNF)-α transgenic mice affected by arthritis display high levels of NGF (3), suggesting that TNF-α is involved in the up-regulation of NGF. More recently, studies carried out on animal models indicated that the increase of NGF in joints of rodents with arthritis is associated with the presence of interleukin (IL)-1β rather than TNF-α, since the administration of the former rather than the latter induces an increase of NGF in the joint (4). To gain further information about the role of these two cytokines and of NGF in RA we measured the levels of these biological markers in the synovial fluids of patients affected by RA.

These studies were carried out using 22 samples of synovial fluid from patients fulfilling the ARA criteria for the diagnosis of RA (mean age 43 years). Synovial fluids of 8 patients affected by non-inflammatory disease osteoarthritis (OA), mean age 67 years, were used as controls. Synovial fluid was drawn during the routine morning analysis (7:00 AM) and quickly centrifuged and stored at -70°C until used for the determinations of NGF, TNF-α and IL-1β. All patients had given their informed consent previously, in accordance with the Helsinki Convention and Italian legislation on biomedical research.

NGF levels were measured by a highly sensitive ELISA which recognizes both murine and human NGF, as previously described (4). All assays were performed in triplicate, and the data are expressed in pg/ml. The recovery of NGF was estimated by adding to the samples a known amount of purified NGF. The exogenous NGF yield was calculated by subtracting the amount of this NGF from the amount of the endogenous variety. Under these conditions the recovery of NGF in our assay ranged from 80 to 90%.

TNF-α activity in the synovial fluid was determined by a cytotoxicity assay using the TNF-sensitive WEHI 164 clone 13 cell line (5).

IL-1β immunoreactivity was measured by means of a commercial immunoenzymatic assay (Quantikine Immunossay for Human IL-1β, R&D System, Minneapolis, USA). For the assay procedure we followed the manufacturer’s instructions.

The data were analysed by means of analysis of variance using the SuperANOVA package for Macintosh (Abacus Concepts Inc., Berkeley, CA, USA). The differences between groups were determined by the non-parametric Mann-Whitney U test; a p < 0.05 was considered statistically significant.

Consistent with previous reports (2) the present findings show that the synovial fluid of patients affected by RA express elevated NGF levels compared with the non-inflammatory synovial fluid of patients with OA (78.8 ± 25.3 pg/ml versus 5 ± 4 pg/ml). The amounts of TNF-α and IL-1β in patients with RA were both significantly augmented when compared with the synovial fluid of patients with OA. The cytotoxicity assay showed that the levels of biologically active TNF-α increased from 0.821 ± 0.6 pg/ml to 13.5 ± 4 pg/ml (p < 0.05). Moreover, ELISA revealed that the levels of IL-1β significantly increased in the synovial fluid of patients with RA compared with those of patients with OA (460 ± 69 pg/ml versus 42 ± 13 pg/ml; p < 0.05). Though the TNF-α evaluation was based on a cytotoxic assay and IL-1β on an immunoenzymatic assay and therefore the concentrations revealed could have been over- or under-estimated, the data obtained fully support the hypothesis of a diverse role of these two cytokines on NGF synthesis and most probably on the pathogenesis of RA.

The present findings show that the synovial fluid of patients with RA is characterized by an up-regulation of NGF associated with an elevated presence of TNF-α and IL-1β. This observation is in line with in vitro findings that these cytokines promote NGF synthesis (6). The present study, carried out in humans, failed, however, to confirm previous studies conducted in an animal model which found that IL-1β but not TNF-α enhances synovial NGF levels (4). How can these different effects be explained?

One possible explanation is that the effect of the exogenous administration of TNF-α can only partially or synergistically promote NGF synthesis, or alternatively that synovial NGF levels are differentially regulated in rodents and humans. Another difference that should be taken into consideration is that, unlike the animal models, human RA is characterized by a persistently elevated presence of cytokines in the synovial fluids (7). Furthermore, patients with RA are exposed to pharmacological treatments that can directly or indirectly influence the synthesis of NGF. Indeed, the basal levels of both NGF and cytokines can be affected by the corticoid hormones used in therapy, as suggested by the observation that patients treated with corticosteroids display decreased levels of NGF and IL-1β in the synovial fluid (unpublished observations).

Therefore, although the mechanisms implicated in the dysregulation of NGF in humans or in animal models of arthritis require additional studies, the present findings clearly indicate that this molecule is involved in the pathogenesis of RA. Altered levels of NGF have been found in juvenile arthritis (JA), psoriasis and spondylarthropathy (SpA) patients (8), further suggesting the potential role of NGF in these inflammatory pathologies. Despite the evidence that JA, RA, psoriasis and SpA are characterised by a dysregulation of the synthesis and/or uptake of NGF, the functional significance of NGF in these diseases is not clearly known. We have recently suggested that NGF may be associated with repair and remodelling mechanisms (3, 4).

As previously reported, our findings support

![Fig. 1. NGF (A), TNF-α (B) and IL-1β (C) levels in the synovial fluids of patients affected by non-inflammatory disease osteoarthritis (OA) and rheumatoid arthritis (RA). *p < 0.05 according to Mann-Whitney U test.](image-url)
the hypothesis that either NGF and/or NGF-antibodies, depending on the physiopathological stage of the disease, could contribute to the development of therapeutic strategies for this inflammatory joint disease.

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HIV infection-associated rheumatic syndromes

Sir,

We read with great interest the Letter to the Editor by Olive et al. “Vasculitis and oral and genital ulcers: Behçet’s syndrome or HIV infection” (1). In general, we agree with their position regarding this association. In our original report (2) we described a patient who exhibited oral ulceration secondary to vasculitis, but who did not fulfill the diagnostic criteria for Behcet’s.

To date, we have evaluated over 1000 HIV-infected individuals and have yet to find a patient fulfilling the diagnostic criteria for Behcet’s disease. Therefore, this leads us to conclude that although the same HIV patients may exhibit vasculitis and genital and oral ulcerations, the association with Behçet’s disease is extremely rare. Our experience is, however, at variance with the authors’ initial statements regarding the frequency and type of rheumatic syndrome(s) seen in the HIV population. Data from our group and others have conclusively shown that rheumatic complaints are relatively common in HIV patients and, contrary to Olivé et al.’s affirmation, Reiter’s syndrome and undifferentiated spondyloarthropathy (reactive arthritis) are the most common rheumatic syndromes seen (3, 4).

Some geographic variation in the frequency and pattern of joint involvement in HIV patients has been noticed, particularly in Spain. This, however, appears to be due to the risk factors involved, usually I.V. drug abuse as in the case of the patient described by Olivé et al. Recent observations on the black races of sub-Saharan Africa have linked HIV infection and seronegative spondyloarthropathy. In their prospective study Njovu et al. (5) found reactive arthritis to be the most common inflammatory joint disorder in black Zambians. They estimated the prevalence of undifferentiated spondyloarthropathy and Reiter’s syndrome to be 180/100,000 in the HIV-positive population and 150/100,000 in the HIV-negative population. Thus, considering that reactive arthritis in Africa was rare prior to the advent of the HIV epidemic, this recently reported data provides strong support for the association of inflammatory arthritic disorders and HIV infection.

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Letters to the Editor

Sirs,

We agree with the comments of Cuellar and Espinoza. Behçet’s syndrome is extremely rare in the setting of HIV infection, although the association is controversial, a fact which is emphasized in our paper (1).

To date we have evaluated in our center 1,350 HIV-positive patients (from 01/98*p992X to9X31/12/1998). Seventy-six patients (5.6%) had osteoarticular manifestations (63 males, 13 females; mean age 34.15 years, SD 9.3). Intraavenous drug abuse was present in 53 (68%). Septic arthritis was present in 21 (27%), soft tissue infections in 11 (14%), lymphoma in 7 (9.2%) and spondyloarthropathies in 7 (7.2%). The different spondyloarthropathies were psoriatic arthritis (n = 3), reactive arthritis (n = 1), Reiter’s syndrome (n = 1) and undifferentiated spondyloarthropathy (n = 2).

Our results are fairly similar to those of other authors from our geographical area, namely, that septic arthritis is the most common osteoarticular manifestation in HIV-positive patients in Spain. This is probably related to practices leading to HIV infection (2-4).

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References