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BRIEF REPORT

Tumour necrosis factor-alpha levels in aqueous humour and serum from patients with uveitis: the involvement of HLA-B27

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SUMMARY

Objective: To study the local and systemic levels of the tumour necrosis factor-α in patients with active uveitis and to determine the implication of TNF-α in rheumatological uveitis and to observe if this relationship is more significant in the B27 positive patients.

Patients and methods: Patients were selected on the basis of a diagnosis of uveitis of any aetiology. Data from 23 patients were stratified into two categories according to the presence or absence of systemic rheumatic disease. The first group comprised nine patients with rheumatic disease; the second group contained 14 patients without rheumatic disease. The patients were also sub-classified into those who were HLA-B27 positive (14 patients) and those who were not. TNF-α levels in serum and aqueous humour from a group of 16 patients with uncomplicated cataracts were analysed as a control group.

Results: In the control group ($n = 16$) the serum TNF-α concentration was 13.1 ± 2.9 pg/ml and the aqueous humour concentration of TNF-α was 0.56 ± 1.53 pg/ml. In uveitis patients ($n = 23$) the serum TNF-α concentration was 35.35 ± 26.77 pg/ml and the aqueous humour concentration of TNF-α was 15.1 ± 1.70 pg/ml ($p < 0.01$). In HLA-B27 positive patients ($n = 9$) the serum TNF-α concentration was 45.56 ± 34.17 pg/ml and the aqueous humour concentration of TNF-α was 15.89 ± 0.93 pg/ml. In HLA-B27 negative patients ($n = 14$) the serum TNF-α concentration was 28.79 ± 19.38 pg/ml and aqueous humour concentration of TNF-α was 14.57 ± 1.91 pg/ml ($p < 0.01$).

Conclusions: The concentration of TNF-α in aqueous humour in patients who are HLA-B27 positive is significantly greater than in those who are B27 negative. No significant differences in the concentrations of TNF-α in serum or aqueous humour in patients with or without rheumatic diseases were detected. TNF-α is a cytokine that may participate actively in the pathogenesis of clinical uveitis.

Introduction

Inflammation of the iris or the corpus ciliare, known as iritis or anterior uveitis, is frequent in the context of several systemic inflammatory diseases[1] including the spondyloarthropathies[2], mainly in ankylosing spondylitis (AS) and reactive arthritis (ReA), which are strongly associated with HLA-B27. In fact, 90% of patients with AS or ReA and uveitis are B27 positive, but in psoriatic arthritis (PsoA) or inflammatory bowel disease arthritis (IBDA), the association is more variable and generally lower.

Although the precise pathogenic mechanisms underlying uveitis have not been fully identified, cytokines appear to be involved in intraocular inflammation and high levels of diverse cytokines in the ocular tissues[3] or the aqueous humour (AH)[4] in uveitis patients have been found. Recently, an active role of TNF-α in the pathogenesis of intraocular inflammation and uveitis in several experimental models in the rat has been demonstrated[5]. TNF-α inhibition by etanercept or infliximab has shown to be effective in uveitis control in several cases[6,7].

We recently demonstrated significantly higher levels of TNF-α in the aqueous humour and sera of patients with a variety of forms of uveitis[8]. In the present study, we attempted to determine the behaviour of TNF-α and its correlation with HLA-B27 by determining its levels in the aqueous humour and sera of patients with active uveitis.

Patients and methods

Twenty-three patients with a diagnosis of uveitis were studied. Patients were included in the study if they had a minimum of 5–10 cells per field (1 mm^2) on slit lamp examination, and if they had a clinical history or characteristics which made it necessary or possible to remove aqueous humour for diagnostic or therapeutic purposes. The patients were divided according to HLA-B27 status: nine patients were HLA-B27 positive (five ankylosing spondylitis, one idiopathic uveitis, two Behçet's disease and one birdshot retinochoroidopathy) and 14 HLA-B27 negative patients(six idiopathic uveitis, two Fuchs's uveitis syndrome, one *Candida* septicaemia, one Behçet's disease, one chronic juvenile iridocyclitis, one lens-induced uveitis, one systemic toxoplasmosis and one graft-versus-host disease). The uveitis group was classified according to the recommendations of the International Group for the Study of Uveitis[9]. Sixteen patients who had been operated on for uncomplicated cataracts served as the control group. None of them had any other disease or history of uveitis.

Aqueous (0.2–0.3 ml) and peripheral blood (10 ml) samples were taken from each patient before the start of steroid therapy. The aqueous humour was extracted under a surgical microscope via limbic paracentesis using a 27-gauge needle. Before the administration of any systemic drugs, peripheral blood was extracted at the time of collection of the ocular specimen. The aqueous humour was deposited in an Eppendorf tube (Eppendorf, Hamburg, Germany) for subsequent processing. The cells were isolated from the supernatant by centrifugation at 3500 rev/min and both components were stored at –70 °C until assay. The principles of the declaration of Helsinki were followed. The Research Standards Committee of our centre approved this project (Resolution No. 68/95). All patients and controls gave their informed consent after the nature of the study had been fully explained to them.

Levels of soluble TNF-α were measured in serum and aqueous humour supernatants. A commercial immunoenzymatic ELISA Kit (Bender Medical Systems, Austria) was used. Serum and aqueous humour samples were diluted 1 : 20. Briefly, 50 µl samples or standard controls were added to wells containing absorbed anti-TNF-α monoclonal antibody. After incubation and washing following the kit protocols, a horse-radish peroxidase (HRP)-conjugated anti-TNF-α antibody was added and bound to the TNF-α captured by the first antibody. Following incubation, any unbound conjugate was removed during a wash step and a substrate solution reactive with HRP (TMB) was added to the wells. A coloured product was formed in proportion to the amount of TNF-α present in the sample. The reaction was finished by addition of acid and the absorbance was measured at 450 nm as the primary wavelength and 620 nm as the reference wavelength. A standard curve was prepared from seven TNF-α standard dilutions and the TNF-α sample concentration was determined. Each determination was carried out in duplicate and the mean of each pair of results was used. The results are expressed in pg/ml and the lower detection limit for TNF-α was 4 pg/ml.

Categorical variables were compared using the χ^2 or Fisher's exact test. The statistical analysis was performed with the SPSS software version 10. The Mann–Whitney non-parametric test for independent samples was used. Pearson's correlation test was used to assess the correlation between two quantitative variables. Statistic significance was considered for every p lower than 0.05.

Results and discussion

The mean values, standard deviations and comparison of TNF-α levels in sera and aqueous humour for 16 cataract patients (control group) and 23 study

Table 1. *Serum and aqueous humour levels of TNF-α*

Patients and controls	TNF-serum (pg/ml)	TNF-aqueous humour (pg/ml)
Control patients ($n = 16$)	13.13 ± 2.92	0.56 ± 1.53
Uveitis patients ($n = 23$)	35.35 ± 26.77*	15.1 ± 1.70†

*Statistical significance determined by Mann–Whitney U test
†Statistical significance determined by Student's *t*-test ($p < 0.01$)

Table 2. *Serum and aqueous humour levels of TNF-α*

Patients	TNF-serum (pg/ml)	TNF-aqueous humour (pg/ml)
B27+ ($n = 9$)	45.56 ± 34.17	15.89 ± 0.93
B27– ($n = 14$)	28.79 ± 19.38*	14.57 ± 1.91†

*Statistical significance determined by Mann–Whitney U test
†Statistical significance determined by Student's *t*-test ($p < 0.01$)

patients are shown in Table 1, and the comparison between HLA-B27 positive and negative patients in Table 2 (nine HLA-B27 positive and 14 HLA-B27 negative).

Our study shows that the aqueous humour and sera from patients with uveitis have higher levels of TNF-α than control patients. These data suggest that TNF-α may be a mediator in ocular inflammation and could be considered as a specific target in the pathogenic pathways.

This higher concentration of TNF-α could explain the acceptable response to anti-TNF-α therapy in primary uveitis or in secondary uveitis in systemic diseases[10–12]. In our patient group we also confirmed a significant increase in TNF-α concentration in the aqueous humour of HLA-B27 positive patients compared to negative ones ($p = 0.039$).

It was interesting that our patients with uveitis showed higher levels of TNF-α in the serum than in the aqueous humour (independently of their HLA-B27 status), suggesting the existence of leakage from the blood to the aqueous humour rather than local production.

It would also be relevant to know if there is a direct relationship between HLA-B27 and TNF-α in rheumatic uveitis development with respect to non-rheumatic uveitis. We did not find any studies that analysed these parameters, even though there is a clear relationship between this class I allele and ocular and osteoarticular inflammation[13,14].

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