Anti-αB-crystallin immunoreactivity in inflammatory nervous system diseases

Abstract αB-Crystallin, a small heat shock protein, is an immunodominant antigen with increased tissue expression in demyelination. To investigate the humoral response against αB-crystallin, the sera and CSF samples of patients with multiple sclerosis (MS), Guillain-Barré syndrome (GBS), neuro-Behçet’s disease (NBD) and other non-inflammatory neurological diseases (NIND) were screened by enzyme-linked immunosorbent assay for anti-αB-crystallin IgG and IgM antibodies. Serum and CSF IgG antibody responses to αB-crystallin were significantly elevated only in NBD patients (serum IgG, NBD 1.29±0.49 vs. NIND 0.95±0.39, P=0.01; CSF IgG, NBD 1.22±0.64 vs. NIND 0.81±0.35, P=0.01). Similarly, high serum IgM antibody titres were also detected in NBD (1.83±0.72 vs. 1.16±0.49, P=0.0005) and in MS (1.57±1.07, P=0.046), whereas elevated CSF IgM responses were observed only in GBS (2.09±1.09 vs. 1.41±0.7, P=0.007). Humoral responses against αB-crystallin are increased in NBD and GBS, which may implicate this central nervous system antigen in the causation and pathogenesis of these inflammatory nervous system disorders.

Key words αB-Crystallin · Neuro-Behçet · Multiple sclerosis · Guillain-Barré syndrome · Antibodies

Introduction

Several lines of evidence suggest a role of an autoimmune response in the pathogenesis of inflammatory nervous system diseases. Attempts to identify the putative antigens specific for the nervous system have yielded a number of candidates such as myelin basic protein, proteolipid protein, P2 and myelin-oligodendrocyte glycoprotein [17]. Heat shock proteins (HSPs), which are highly homologous in various species and induced in a wide variety of cells in response to stress, have also been investigated, and the question has been asked whether HSPs can also be involved in the development of autoimmune process [23]. Numerous HSP families, categorized according to their molecular weights have been found to be related to different experimental and human diseases. Antibody titres against human HSP60 are found to be significantly elevated in the CSF of a subgroup of multiple sclerosis (MS) patients [14]. In addition, mycobacterial HSP70 reactive T cells have also been shown to be more frequent in the peripheral blood of MS patients [16].

αB-Crystallin has been shown to be a candidate autoantigen sharing sequence homologies in various species and functioning as an HSP. The protein is abundant in vertebrate eye lens and found in several other organs including skeletal muscle and kidney epithelial cells [6]. Stress-induced expression of αB-crystallin in the central nervous system has been reported to be elevated in a variety of pathological conditions, including inflammation and demyelination [18]. In this study, we aimed to investigate the humoral response against αB-crystallin in patients with inflammatory nervous system diseases and determine if this protein can be considered as a potential antigen in these conditions.
system (CNS) glia cells has also been demonstrated [11]. The search for nervous system specific autoantigens has shown that αβ-crystallin is an immunodominant antigen to human T cells when expressed at the elevated levels, such as in MS lesions [19, 20].

Behçet’s disease (BD) is a systemic vasculitis of unknown cause with mucocutaneous, ocular and vascular manifestations. Diffuse parenchymal or vascular involvement of the CNS is a distinct form of the disease, namely neuro-Behçet’s disease (NBD) [2–4]. Possible autoimmune responses against mycobacterial HSP65 and human HSP60 have been demonstrated in BD suggestive of their role in the etiopathogenesis [7, 8, 12, 13]. An increased local humoral immune response against mycobacterial HSP65 is also shown in the CSF of patients with parenchymal NBD [18].

The involvement of αβ-crystallin has been implicated in the pathogenesis of MS [1], but data on its role in other inflammatory nervous system diseases are lacking. In this study we examined the systemic and local humoral immune response to αβ-crystallin in patients with various inflammatory diseases of central (MS) or peripheral (Guillain-Barré syndrome GBS) nervous system, in a systemic inflammatory disease with neurological involvement (NBD) and in other non-inflammatory neurological diseases (NIND).

### Material and methods

**Patients and samples**

We studied 27 NBD patients (22 men, 5 women, mean age 40.7 years) followed at the Neurology Department of the University of Istanbul Medical Faculty, classified according to the International Study Group criteria [10]. Of the 27 patients 20 (16 men, 4 women) had cerebral (n=17) or medullary (n=3) parenchymal involvement (P-NBD); 7 patients (6 men, 1 women) were diagnosed with intracranial hypertension (IH-BD) [4]. All patients had recurrent oral and genital ulcers, 24 (89%) had mucocutaneous, 17 (63%) articular and 13 (48%) ocular manifestations.

Also examined were 33 patients with MS (11 men, 22 women, mean age 38.5 years) classified according to the Poser et al. [15] criteria as 25 with relapsing-remitting, 5 with secondary progressive and 3 with primary progressive clinical course. The mean disease duration of the MS patients was 8.5 years. A further 33 patients with GBS (21 men, 12 women) and 25 patients with NIND (10 men, 15 women) were investigated. Their diagnoses were: lumbar disc protrusion (CSF taken during myelography; n=2), acute psychotic disorder (n=2), headache (n=4), idiopathic epilepsy (n=4), benign intracranial hypertension (n=1), senile dementia (n=4), Alzheimer disease (n=1), spinal muscular atrophy (n=2), transient ischaemic attack (n=1) and genetic defects (n=4). All lumbar punctures were carried out for diagnostic purposes.

**Enzyme-linked immunosorbent assay (ELISA)**

Aliquot CSF and serum samples were stored at –80 °C until studied. IgG and IgM antibodies against αβ-crystallin were investigated by enzyme-linked immunosorbent assay in paired CSF and serum samples. Plates (Nunc, Maxisorp, Denmark) were coated with 100 µl 5 µg/ml αβ-crystallin isolated from bovine eye lens as previously described [21] and incubated overnight at 4 °C. Bovine αβ-crystallin is highly homologous with the human with four amino acid differences. After washing with 1% Tween-20 in phosphate-buffered saline (PBS 20), the plates were blocked with PBS 20 and 5 % dried milk (5% PBS 20) at 37 °C for 1h. Then the serum samples, diluted to 1:100 in 0.5% PBS 20 (100 µl per well) and undiluted CSF samples (50 µl per well) were added in duplicate. The samples were incubated at 37 °C for 2h. After extensive washing 100 µl peroxidase-conjugated specific anti-human IgG, diluted to 1:10000 or IgM, diluted to 1:3000 was added to each well (DAKO, Denmark) and the plates were incubated at room temperature absorbance values were read at 492 nm. The samples were investigated blindly. A standard control pool of four sera known to be negative and CSF from a patient with congenital hydrocephalus was run at each assay. The results were calculated by dividing the sample OD by the standard control OD and given as ELISA ratios. The specificity of ELISA is confirmed with anti-αβ-crystallin antibodies in inhibition assays. Antibodies against HSP65 were determined as previously reported [18].

**Statistical methods**

Intergroup ratios were analysed by one-way analysis of variance and comparisons between individual groups were analysed with the Mann-Whitney U test. Correlations between serum and CSF ELISA ratios were analysed by using Spearman’s rank test.

### Results

**Serum responses**

As shown in Table 1, we detected a significant IgM response against αβ-crystallin in the sera of NBD patients with a mean ELISA ratio of 1.83±0.72, compared with 1.18±0.5 in GBS (P=0.0002), 1.57±1.07 in MS (P=0.01) and 1.6±0.49 in NIND (P=0.0005; Fig. 1). The IgM response in MS was also significantly higher than in GBS (P=0.04) and in NIND (P=0.046) groups. Comparing the IgG results, we have again shown a prominent response to αβ-crystallin in the sera of NBD patients (Fig. 1). The ELISA ratios detected in the NBD group with a mean of 1.29±0.49 were significantly higher than those all other groups: GBS (0.80±0.36, P=0.0001), MS (0.7±0.32, P<0.0001) and NIND (0.95±0.39, P=0.01).

**CSF responses**

In the CSF an increased IgM response was shown only in GBS patients (Fig. 1). The mean ELISA ratio of 2.09±1.09 in this group was significantly higher than NBD (1.2±0.35, P=0.0004), MS (1.17±0.48, P<0.0001) and NIND (1.4±0.7, P=0.007) groups. A weak correlation was observed between the CSF protein content and anti-αβ-crystallin reactivity in GBS patients (r=0.43, P=0.05).

The IgG response to αβ-crystallin was shown to be higher in the CSF of NBD patients than in that of other