T cell response to human HSP60 and yersinia 19 kDa in ankylosing spondylitis and rheumatoid arthritis: no evidence for a causal role of these antigens in the pathogenesis

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The pathogenesis of two important inflammatory rheumatic diseases, rheumatoid arthritis (RA) and ankylosing spondylitis (AS), is not clear. In both diseases an immune response against an unknown autoantigen may have a crucial role.^{1,2} It has been repeatedly suggested that heat shock proteins (HSP) play a part in various autoimmune diseases such as RA, diabetes, and multiple sclerosis,³ based on high interspecies sequence homologies, inducible tissue expression, and a strong immunogenicity. On the other hand, some studies indicate that HSP may even be protective in arthritis.⁴

The 19 kDa urease β subunit of yersinia is regarded as a major immunodominant protein for both T cell and antibody responses in patients with yersinia induced reactive arthritis.⁵ Because 20–30% of HLA-B27+ patients with yersinia induced

reactive arthritis develop the full picture of AS after 10–20 years,⁶ it is an obvious question to ask whether a T cell response against the yersinia-specific 19 kDa protein is also detectable in patients with AS. Until now, there has been insufficient evidence to support a relationship between yersinia infection and RA.

PATIENTS AND METHODS

In this study 45 patients with active AS and 22 with active RA were recruited from the outpatient clinic of the University Hospital Benjamin Franklin, Berlin. In vitro stimulation of CD4+ T cells by protein antigen was carried out for six hours, as described previously.^{7,8} Brefeldin A (10 μ g/ml; Sigma) was

Figure 1 Examples of the antigen-specific response to the human (h)-HSP60 or yersinia (Y)-19 kDa compared with stimulation without antigen, in a patient with ankylosing spondylitis (AS) or rheumatoid arthritis (RA) or a healthy control (HC). After staining for T cell surface markers and intracellular cytokines a gate for CD4+ T cells was set. The percentage of IFN γ /CD69 double positive cells of the CD4+ T cell subpopulation is indicated.

Figure 2 Percentage of patients with ankylosing spondylitis (AS, n=45), with rheumatoid arthritis (RA, n=22), and of healthy controls (HC, n=20) responding to in vitro stimulation with the human heat shock protein 60 (h-HSP60) or yersinia 19 kDa (Y-19 kDa). Response was measured either by IFN γ or TNF α production of CD4+ T cells after antigen-specific stimulation in comparison with stimulation without antigen. The analysis was made with whole peripheral blood.

added for the last four hours of the stimulation for accumulation of intracellular cytokine. After fixation with 2% formaldehyde, the cells were quadruply stained for CD4, CD69 surface marker, and for the intracellular cytokines interferon γ (IFN γ) and tumour necrosis factor α (TNF α).^{7,8} After gating on CD4+ T cells, only cytokine positive T cells which were also positive for the early activation antigen CD69 were counted.

RESULTS

After stimulation with staphylococcal enterotoxin B the percentage of positive cells was always >8%, whereas after stimulation with no antigen the percentage was always <0.01%. Samples with more than 0.05% CD69/cytokine double positive cells out of the CD4+ T cell population were regarded as positive.^{7,8} Figure 1 shows examples of T cell responses to human (h)-HSP60 and Y-19 kDa in one patient with AS, one with RA and a healthy control (HC).

There was no difference between the patients with AS, those with RA, and healthy controls (fig 2) in the peripheral blood T cell response to the h-HSP60 as judged by the percentage of CD69/IFN γ or CD69/TNF α double positive CD4+ T cells.

For synovial fluid (SF), 5/7 (71%) patients with AS and 3/4 (75%) patients with RA showed a T cell response to h-HSP60 by IFN γ secretion. Six of seven (86%) patients with AS and 3/4 (75%) with RA showed a TNF α secretion in response to h-HSP60. In comparison with peripheral blood, the h-HSP60-specific IFN γ /TNF α responses were stronger in SF (mean (SD) 0.18 (0.3) in SF v 0.09 (0.08) in peripheral blood for IFN γ ; 0.35 (0.64) in SF v 0.15 (0.05) in peripheral blood for TNF α), but this difference was not significant ($p>0.05$ for both cytokines).

As judged by the percentage of CD69/IFN γ double positive CD4+ T cells, a similar T cell response to the Y-19 kDa was also found in peripheral blood in all three groups (fig 2). Although a higher percentage of patients with AS (3/7 (43%) patients for IFN γ ; 5/7 (71%) patients for TNF α) than with RA (1/4 (25%) patients for IFN γ ; 2/4 (50%) patients for TNF α) showed a synovial T cell response to the 19 kDa protein; this difference was non-significant, possibly owing to the small number of patients.

DISCUSSION

These results suggest that T cell responses to h-HSP60 and to Y-19 kDa are present in all three groups and seem therefore

not to be pathogenic. We cannot exclude the possibility that T cells specific for one of the two antigens are present in synovium and just are not detected in peripheral blood. However, the absence of clear differences between antigen-specific T cells in the SF of patients with RA and AS argues against this, unless a similar pathogenesis for these two diseases is assumed, which is unlikely.

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