The 30-kDa band from *Salmonella typhimurium*: IgM, IgA and IgG antibody response in patients with ankylosing spondylitis

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**Objective.** To determine the association of *Salmonella typhimurium* antigens with AS by analysing the IgA, IgG and IgM antibody response to the crude lysate and the 30-kDa band from this micro-organism.

**Methods.** Sera from 28 AS patients, 28 HLA-B27+ healthy relatives, 28 unrelated healthy subjects and 14 RA patients were included. *Salmonella typhimurium* proteins were electrophoretically separated and blotted onto nitrocellulose sheets for immunodetection with sera from AS patients and unrelated healthy subjects. The electroeluted 30-kDa band (p30) and a crude lysate (SIcL) from *S. typhimurium* were used as antigen to evaluate the IgM, IgA and IgG (total and subclasses) antibody levels by ELISA. An inhibition assay was carried out to confirm the specificity of IgG response to the p30.

**Results.** Twenty out of 28 AS patients (71.4%) and 4 out of 28 unrelated healthy subjects (14.3%) recognized a 30-kDa band from *S. typhimurium* with IgG antibodies. Six out of 28 AS patients (21.4%) and 4 out of 28 unrelated healthy subjects (14.3%) detected it with IgA antibodies. Recognition of p30 and SIcL by both IgA and IgG antibodies was higher in AS patients than in control groups (P<0.003, 0.001 and 0.03 for IgA and 0.003, 0.003 and 0.006 for IgG). Sera from AS patients have higher percentage of IgG antibodies p30 and IgG3 subclass was higher in AS patients than in control groups. No differences in the IgM response were found.

**Conclusions.** Data presented suggest the association between the p30 and AS.

**Key words:** Ankylosing spondylitis, *Salmonella typhimurium*, Antibody levels, Autoimmunity.

**Introduction**

AS is the major subtype and a main outcome of an inter-related group of rheumatic diseases now named SpAs. AS is a chronic inflammatory disease of unknown aetiology, in which immunogenetic and environmental factors are involved [1]. The former is represented by the association with genes of the MHC, mainly HLA-B27 [2–4]. Further support for a common genetic background comes from the existence of borderline patterns of SpA and from the presence in some families of members with different SpA subtypes. Family clustering is a striking feature that emphasizes the existence of genetic susceptibility factors [5].

Environmental factors are also involved in pathogenesis of SpA. The fact that ReA is triggered by genitourinary infections with *Chlamydia trachomatis* or by enteritis by Gram-negative bacteria such as *Shigella* and *Salmonella*, *Yersinia* and *Campylobacter* provides a solid background for the possible interaction between HLA-B27 and bacteria, but the evidence for triggering infection in other SpAs, including AS, is marginal [6, 7]. Thus, SpAs are multifactor-related diseases whose pathogenesis involves gene–environment interactions [5, 6, 8, 9]. Studies on the association between AS and bacterial infections has been reported. The immune response to enterobacteria such as Klebsiella pneumoniae, *Salmonella typhimurium*, *Shigella flexneri* and *Yersinia enterocolitica* has been evaluated and these bacteria have been associated with AS [9–15]. Most studies have been carried out with *K. pneumoniae* [16–18]; however, it’s dominant role in AS has not been confirmed [12].

In this context, it has been reported that the behaviour of *S. typhimurium* was modified in HLA-B27 U9811-transfected cells [19]; particularly, the infection by *Salmonella* induces more IL-10 and lower TNF-α synthesis in the response of infected cells. Also, *Salmonella* sp. DNA was detected in SF from patients with SpA [20]. On the other hand, lymphoproliferative response of mononuclear cells from SF in the presence of *S. typhimurium* was also reported in patients with ReA and uSpA [21].

The antibody response to *S. typhimurium* in AS patients has been analysed by some authors [10, 12, 22–24]. However, all these studies have been made with the hypophilized bacteria or crude lysate from the bacterium. These studies emphasize the association of this bacterium with AS, but a particular antigen towards which the immunological response is directed in AS patients and could then be involved in the pathogenesis of the disease, has not yet been reported. The aim of this work was to identify the particular antigens from sonicated crude lysate from *S. typhimurium* (SIcL) recognized by the humoral immune response in AS patients that could be involved in the immunopathogenesis of the disease.

**Materials and methods**

**Patients and controls**

We included 28 consecutive patients with AS [25] attending the outpatient clinic of our department. As control group, 28 HLA-B27+ healthy relatives of AS patients, 28 unrelated healthy subjects and 14 patients with RA were included. All participants were informed about the nature of the study and written consents were obtained according to the Declaration of Helsinki. They were bled by venipuncture and the serum was obtained. The study was approved by the local ethics committee.

**Antigens**

**Bacterial strain.** A typical *S. typhimurium* strain was kindly donated by Laboratorio Estatal de Salud Publica from...