**SUMMARY**

IgA1 and IgA2 subclass serum antibodies against whole *Klebsiella pneumoniae* bacteria were studied earlier in the sera of 98 patients with ankylosing spondylitis (AS) and in 100 healthy blood donors by enzyme immunoassay. In this study, the patients were divided into groups according to the clinical picture, i.e. the presence or absence of iritis and enthesitis. The previous findings of increased IgA1 and IgA2 subclass antibody levels against *K. pneumoniae* in AS patients when compared to the healthy controls were not specifically associated with any single AS patient group in the present study, but instead were similarly seen in all patient groups with/without extra-articular features. This is in line with the previous studies suggesting a role for *K. pneumoniae* in the pathogenesis of AS.

**KEY WORDS:** Ankylosing spondylitis, Antibodies, *Klebsiella pneumoniae*, IgA1 and IgA2 subclasses, ELISA, Iritis, Enthesitis.

The association between ankylosing spondylitis (AS) and intestinal infection with *Klebsiella pneumoniae* and possession of the HLA-B27 antigen have received much attention [1, 2]. There are reports of increased faecal carriage of *Klebsiella*, as well as reports of increased serum antibody levels against *Klebsiella* bacteria in patients with AS [3–7]. Recently, we showed that the increased *K. pneumoniae*-specific IgA antibody level in AS patients consisted of both IgA1 and IgA2 subclasses [8].

Iritis or acute anterior uveitis is a well-known clinical entity in AS. It is usually acute, self-limiting and unilateral. It is held that some 25% of patients with AS will be affected with this complication during the course of their disease [9]. Interestingly, over 50% of patients diagnosed as having acute anterior uveitis carry HLA-B27 [10]. Enthesitis is another extra-articular feature occasionally associated with AS.

Little is known about the immunological features of iritis in patients with AS. It has been shown, however, that in patients with HLA-B27-positive acute anterior uveitis the total serum IgA antibody level is increased [11, 12]. Also the IgA-containing immune complex level has been shown to be increased in the sera of patients with acute anterior uveitis [12]. Further, the occurrence of serum antibodies against *K. pneumoniae*, *Escherichia coli*, *Proteus mirabilis* and Yersinia has been reported to be increased in patients with acute anterior uveitis when compared to healthy controls [13, 14].

Recently, we showed that AS patients with iritis had higher IgA class antibody levels against *K. pneumoniae* and *E. coli* lipopolysaccharides when compared to the AS patients without this complication [15]. In addition, the patients without enthesitis had higher IgG class antibody levels against whole *K. pneumoniae* bacteria when compared to the patients with enthesitis [15]. To study the specific role of *K. pneumoniae* in the pathogenesis of AS patients with iritis and enthesitis further, we have now analysed the serum IgA subclass antibody levels against whole *K. pneumoniae* bacteria by enzyme-linked immunosorbent assay (ELISA) in AS patients with/without iritis and enthesitis.

**PATIENTS AND METHODS**

**Patients and serum samples**

Patients consisted of 98 people with AS (25 females and 73 males) with a mean age of 39 yr, ranging from 18 to 67 yr [6]. The clinical picture in 29 patients included iritis (either at the time when the blood sample was drawn or in the past; diagnosed by an ophthalmologist) and in 22 patients it included enthesitis (also at present or in the past; the clinical diagnosis was made by a rheumatologist). There were no differences between the usage of non-steroidal anti-inflammatory drugs or in other anti-rheumatic drugs between the groups. The patient population included 36 patients with the sum of values for erythrocyte sedimentation rate (ESR; normal range 1–10 mm/h) and C-reactive protein (CRP; normal range <10 mg/l) ≤30 and 62 patients with the same value > 30 [6]. All sera, including control sera from 100 healthy blood donors (73 females and 27 males; mean age 38 yr, range 17–63), were stored at −20°C until tested simultaneously.

**Klebsiella pneumoniae antigen for ELISA**

Sodium dodecyl sulphate (SDS) extract of whole *K. pneumoniae* bacteria (ATCC 27736) was used as antigen in ELISA. To prepare antigen, 10 ml of an...
overnight nutrient broth culture of *K. pneumoniae* were diluted 1:40 in fresh nutrient broth and grown on a shaker for 4 h at 37°C. After three washings, the bacteria were treated with 0.1% (weight/volume) SDS for 1 h at 37°C to obtain an antigen extract (SDS-extract).

**ELISA for *K. pneumoniae*-specific antibodies of IgA1 and IgA2 subclasses**

*Klebsiella pneumoniae*-specific antibodies of the IgA subclasses, IgA1 and IgA2, were measured as described earlier for Yersinia and *Salmonella* antibodies [16, 17]. Serum samples at 1:50 dilution in 1% bovine serum albumin (BSA) in phosphate-buffered saline (PBS; 0.1 mol/1, pH 7.5) were allowed to react with the SDS-extract antigen of *K. pneumoniae* (0.1 μg/ml) attached to polystyrene microtiter plates (Nunc, Roskilde, Denmark) overnight at room temperature. Monoclonal antibody against IgA1 or IgA2 (Becton Dickinson, Mountain View, California) was added at a dilution of 1:100. After incubation for 3 h at 37°C, rabbit anti-mouse immunoglobulin (DAKOpatts a/s, Glostrup, Denmark) was added at a dilution of 1:100 and incubated for 3 h at 37°C. Swine alkaline phosphatase-conjugated anti-rabbit IgG (Orion Diagnostica, Espoo, Finland) at a dilution of 1:250 was added to detect bound antibodies. Fresh *p*-nitrophenyl phosphate diethanolamine-MgCl₂-buffer solution (1 mg/ml; Orion Diagnostica) was added, incubated for 30 min at 37°C and the reaction stopped with 1 mol sodium hydroxide. The optical density was measured with a Titertek Multiscan Photometer (Labsystems, Helsinki, Finland) at a wavelength of 405 nm.

**Statistical analysis**

The mean concentrations of antibodies in different groups were compared with Student's *t*-test.

**RESULTS**

When the patients were grouped according to the presence or absence of iritis in the clinical picture, both patient groups, i.e. the patients with and without iritis, had statistically significantly higher IgA1 and IgA2 subclass antibody levels to *K. pneumoniae* when compared to the healthy controls (Table I). No significant differences were observed in the antibody levels between the patients with and without iritis.

When the patients were grouped according to the presence or absence of enthesitis, the results were similar (Table I). Thus, both patient groups had higher IgA1 and IgA2 subclass antibody levels against *K. pneumoniae* when compared to the healthy controls, but no significant differences were seen between the patient groups with and without enthesitis.

**DISCUSSION**

In the present study, we have shown that when compared to healthy controls, serum IgA subclass antibody levels against *K. pneumoniae* are higher in AS patients regardless of the presence of iritis or enthesitis in the clinical picture. Correspondingly, we showed recently that also in AS patients in general the increased anti-*Klebsiella* IgA antibody response consists of both IgA1 and IgA2 subclasses [8]. The increased IgA1 *Klebsiella*-specific antibody level suggests a non-secretory origin for the increased total serum IgA, which must be ascribed to the central immune system [18-21], whereas the increased IgA2 subclass antibody level suggests a contribution of mucosa, e.g. gut, to the increased IgA production [16, 18-21]. The latter is of special interest, as the relationship of gut to inflammatory joint manifestations has received much attention recently [22-24]. Thus, it is intriguing to speculate what are the mechanisms by which *Klebsiella* bacteria, and/or the antibodies evoked against them, might produce the lesions of the disease. *Klebsiella* infection in the gut may be a causative factor for the previously described inflammation [22, 23] and enhanced permeability of the gut [24] in patients with AS. This could then allow certain antigenic material, such as *Klebsiella* lipopolysaccharide, to pass through the mucosa into the circulation. If the mechanisms for both AS and reactive arthritis are equal, microbial antigens, such as *Klebsiella* lipopolysaccharide, are transported into the sites of inflammation, i.e. axial and peripheral joints, as well as uvea and tenons of these patients [25-27]. After this joint or extra-articular complications are initiated according to e.g. molecular mimicry theory [1, 4], or by a yet unknown mechanism, similarly to that seen in reactive arthritis [25-27]. The finding that *Klebsiella* microorganisms have antigens which partially resemble eyeball components [28] supports this. Consequently, IgA1 and IgA2 class antibodies are produced as a result of mucosal and systemic antigenic stimulation in all AS patients regardless of whether they do or do not also have extra-articular clinical features (as shown in the present study), or whether they have purely axial type of disease or also peripheral joint arthritides [8].

The findings of increased total serum IgA level [11, 12], of increased occurrence of antibodies to *K. pneumoniae*, *E. coli*, *P. mirabilis* and *Yersinia* [13, 14] and of increased faecal carriage of *K. pneumoniae* [28] in patients with acute anterior uveitis are in line with the present findings. On a contradictory note, in another study the levels of IgA or IgG *Klebsiella*
antibodies did not differ between the controls and the HLA-B27-positive or -negative patients with acute anterior uveitis or HLA-B27-positive AS patients with acute anterior uveitis [29]. Further, the increase in the serum level of IgA-containing immune complexes in patients with acute anterior uveitis [12] is of interest, as the deposition of immune complexes in the uvea and the subsequent tissue damage has been suspected to form an important mechanism in the initiation of uveitis [30]. In the light of the present findings, the complexes might contain either IgA1 and/or IgA2 subclasses.

Earlier we have shown that AS patients with iritis have higher total IgA class antibody level against K. pneumoniae and E. coli lipopolysaccharides when compared to AS patients without iritis [15]. However, similarly to the present findings, when the antibodies were measured against the whole K. pneumoniae and E. coli bacteria, the total IgA class antibody level did not differ between the patients with and without iritis [15]. This may indicate a specific role only for K. pneumoniae lipopolysaccharide in the pathogenesis of iritis in patients with AS. Consequently, if the IgA1 and IgA2 subclass antibodies had been measured against pure lipopolysaccharide of K. pneumoniae, the present results might have been different. The measurement of IgA class Klebsiella antibodies containing secretory component might have also given some interesting additional information, as the serum secretory IgA level has been shown to be persistently raised in AS patients [31]. Further, if the recently published important capsular antigens of K. pneumoniae had been used as antigens in the assay, the results might also have been different [32].

In conclusion, the IgA1 and IgA2 subclass antibody levels against the whole K. pneumoniae bacteria are both increased similarly in AS patients with/without iritis and enthesitis, probably as a result of mucosal and systemic antigenic stimulation. This is in line with the previous studies suggesting a role for K. pneumoniae bacteria in the pathogenesis of AS with/without extra-articular features.

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