Association of gut inflammation with increased serum IgA class Klebsiella antibody concentrations in patients with axial ankylosing spondylitis (AS): implication for different aetiopathogenetic mechanisms for axial and peripheral AS?

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Abstract
Objectives—A role for Klebsiella pneumoniae in ankylosing spondylitis (AS) has been suggested because faecal carriage of Klebsiella and serum Klebsiella specific antibodies may be increased in this disease. This study has extended the earlier findings by comparing Klebsiella specific serum IgA class antibodies with inflammatory changes in the gut.

Methods—IgA antibodies to K pneumoniae, Escherichia coli, and Proteus mirabilis in serum samples of 25 patients with AS, of eight control patients, and of 100 healthy blood donors were measured by enzyme immunoassay. Gut inflammation of the patients was studied by ileocolonoscopy.

Results—Increased IgA antibody concentrations to K pneumoniae associated with gut inflammation in patients with axial form of AS. Such association was not seen in patients with peripheral form of AS. Such association was not seen in patients with peripheral form of AS. However, at least some of the patients with axial AS without gut inflammation, as well as patients with peripheral AS did not have increased K pneumoniae antibody concentrations, which may be regarded as evidence against the direct role of K pneumoniae in the pathogenesis of AS. However, at least some of the patients with axial AS without gut inflammation, as well as patients with peripheral AS did not have increased K pneumoniae antibody concentrations, which may be regarded as evidence against the direct role of K pneumoniae in the pathogenesis of AS. This study has extended the earlier findings by comparing Klebsiella specific serum IgA class antibodies with inflammatory changes in the gut in patients with axial and peripheral types of AS may be different.

Conclusions—These findings may provide further evidence for the role of K pneumoniae in the pathogenesis of AS. The aetiopathogenetic mechanisms in the axial and peripheral form of AS may be different.

The aetiology of ankylosing spondylitis (AS) is still unknown but the Gram negative micro-organism Klebsiella pneumoniae has been suggested to play an important part in the pathogenesis, although this association between Klebsiella and AS is still a subject of controversy. A high frequency of inflammatory changes, and increased permeability of the gut, as well as high serum concentrations of total IgA and secretory IgA support the role of gut and mucosal immune defence mechanisms. Furthermore, AS can be divided into two forms: (a) patients who have pure axial form of the disease and (b) patients with not only axial, but also peripheral joint arthritides. There have been suggestions for different aetiopathogenetic mechanisms for these two forms. For example, ileocolonoscopic differences have been reported between patients with axial and peripheral form of AS. In addition, sulphasalazine, a drug used to treat inflammatory bowel disease, seems to be especially effective in patients with the peripheral type of AS. We have extended the earlier findings by comparing Klebsiella specific serum IgA class antibodies with inflammatory changes in the gut in patients with axial and peripheral types of AS.
Gut inflammation and serum IgA class Klebsiella antibody concentrations

Antibodies against inflammation in colon. IgA class serum were normal, except for one patient with mild rheumatic disease. Patients (eight with axial and five with peripheral form of AS) were identified as having chronic inflammation in the gut. IgA class serum antibodies against *Klebsiella pneumoniae*, *Escherichia coli*, and *Proteus mirabilis* were measured by enzyme immunoassay (EIA) as described earlier. Briefly, the polystyrene microtitre plates (Nunc, Roskilde, Denmark) were coated with sodium dodecyl sulphate extracts of the bacteria in phosphate buffered saline (PBS) overnight at 37°C. The plates were saturated with 1% normal sheep serum in PBS (NSS-PBS). Patient serum samples at 1:250 dilution were incubated on the plates for two hours at 37°C. Thereafter, alkaline phosphatase conjugated swine anti-human IgA (Orion Diagnostica, Espoo, Finland), diluted 1:250, was incubated on the plates overnight at room temperature. Fresh p-nitrophenyl phosphate in diethanolamine-MgCl₂-buffer solution (1 mg/ml; Orion Diagnostica) was added, incubated for 30 minutes at 37°C, and the reaction stopped with 1 M sodium hydroxide. The optical density was measured with a TiterTec Multiscan Photometer (Labsystems, Helsinki, Finland) at a wavelength of 405 nm. Antibody concentrations were expressed as enzyme immunoassay units (EIU): 1 EIU is 1/100 of the corresponding antibody concentration in the positive reference serum.

The median concentrations of antibodies in different groups were compared with the Mann-Whitney test.

**Results**

Similar to earlier findings the AS patients had higher IgA class serum antibody concentrations against *K pneumoniae* when compared with the healthy controls (mean (SD) EIU: 20.9 (27.0) and 12.3 (11.4), respectively; p < 0.05). When the patients were grouped as having either axial or peripheral type of AS, this increase was seen only in patients with axial type of the disease (28.7 (33.2); p < 0.001). No statistically significant increases were seen in the IgA antibody values against *E coli* or *P mirabilis* in the AS patients.

When *K pneumoniae* specific serum antibody concentrations were analysed in relation to ileocolonoscopic findings, the patients with axial AS and gut inflammation always had higher average Klebsiella specific IgA class antibody values when compared with the patients without these gut lesions and with the control groups (fig 1). When the Klebsiella antibody concentrations were compared with the patients without a gut lesion and with the control groups, the patients with axial AS had higher antibody values compared with the healthy blood donors (EIU; 1 EIU: 20.9 (27.0) and 12.3 (11.4), respectively; p < 0.05). When the patients were grouped as having either axial or peripheral type of AS, this increase was seen only in patients with axial type of the disease (28.7 (33.2); p < 0.001). No statistically significant increases were seen in the IgA antibody values against *E coli* or *P mirabilis* in the AS patients.

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The association between the increased Klebsiella specific IgA class antibody concentrations and gut inflammation in AS patients with pure axial form of the disease seemed to be specific for *K pneumoniae*, as no such increases were seen in the *P mirabilis* specific antibody concentrations in patients with gut lesions, and only patients with inflammation in colon had increased antibody concentration to *E coli* (p < 0.05) when compared with the blood donors.

Interestingly, the patients with peripheral type of AS and inflammation in ileum
responses. mucosa to stimulate systemic immunity and in larger amounts through the inflamed structures may pass into circulation more easily. This is logical as Klebsiella bacteria and their products may mimic antigens of these microbes. The significantly lower antibody concentrations against *E. coli* in patients with inflammation in colon may have been caused by the close similarity of certain antigens of these microbes. The specifically decreased antibody concentrations against *E. coli* in patients with peripheral AS when compared with the blood donors is difficult to interpret, but this is another dissimilarity between axial and peripheral forms of AS. Furthermore, as suggested already in the 1900s and as continuously discussed today, the aetiopathogenetic mechanisms in the axial and peripheral form of AS may be different.

Discussion

Our results show that the Klebsiella specific IgA class serum antibody concentrations are increased and associated with gut inflammation, but only in patients with pure axial AS. This is logical as the Klebsiella bacteria and their products may pass into circulation more easily and in larger amounts through the inflamed mucosa to stimulate systemic immune responses. *K. pneumoniae* shares short sequence with the major histocompatibility antigen HLA-B27, which is closely associated with AS. Such mimicking could lead to unwanted cross reactivity (autoimmunity) or ineffective clearance (inappropriate tolerance) and thus to changed immune responses and consequently to the persistence of bacterial structures in the body as shown to be the case in another HLA-B27 associated disease, reactive arthritis.

However, mechanisms determining specificity of this phenomenon (association of increased enterobacterial antibody values with inflammation in the gut) primarily to Klebsiella and only to the pure axial form of AS are unknown and hence further studies and with a larger number of patients are warranted.

These findings may provide further evidence for the role of *K. pneumoniae* in the pathogenesis of AS. However, many of the patients with pure axial AS with and without gut inflammation, as well as patients with peripheral AS did not have increased *K. pneumoniae* antibody values, which may be regarded as evidence against the direct role of *K. pneumoniae* in the pathogenesis. The finding of increased antibody concentration to *E. coli* in patients with inflammation in colon may have been caused by the close similarity of certain antigens of these microbes. The significantly lower antibody concentrations against *E. coli* in patients with peripheral AS when compared with the blood donors is difficult to interpret, but this is another dissimilarity between axial and peripheral forms of AS. Furthermore, as suggested already in the 1900s and as continuously discussed today, the aetiopathogenetic mechanisms in the axial and peripheral form of AS may be different.

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Unusual but memorable

Series editor: Gary D Wright

A 40 year old black women presented with pronounced soft tissue swelling of several fingers and toes, with tenderness and restriction of involved interphalangeal joints. A few patches of indurated depigmented skin were also noted. Radiographs revealed gross soft tissue swelling with deforming erosion and resorption of phalanges (figure).

Osseous involvement in chronic sarcoidosis occurs in about 5% of all patients. The most characteristic clinical picture is dactylitis resulting from granulomatous cyst formation in the phalanges where it may be the presenting feature of the disease particularly in black patients.1 Numerous radiographic features have been described including expanded phalanges with thin cortical bone and cyst-like spaces, round or oval punched out areas, and fine lattice-like configuration. Periostial reaction is rare. In severe cases, as in this patient, affected phalanges may be fragmented and resorbed with “telescoping” of the digit. Severe soft tissue swelling may result from granulomatous change in the tendon sheath and joint synovium adjacent to affected bones. Joint destruction and collapse may follow local extension of osseous disease.


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