Sequential studies in ankylosing spondylitis

Association of Klebsiella pneumoniae with active disease

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SUMMARY A study of 163 patients with ankylosing spondylitis seen on 433 occasions showed that active inflammatory disease was strongly associated with the presence of Klebsiella pneumoniae in the faeces (P<0.001). Sequential studies showed that in patients with inactive disease the presence of a positive culture for Klebsiella was associated with the subsequent development of active inflammatory disease (P<0.001). These findings support the hypothesis that Klebsiella pneumoniae may be an initiating agent in ankylosing spondylitis.

There is much indirect evidence for an infective aetiology in ankylosing spondylitis (AS). Patients with the disease often experience marked fluctuations in symptoms and disease activity, which have many of the hallmarks of response to an infection. The disease may present as an undiagnosed pyrexia and constitutional disturbances can often be severe. However, no causal agent has been definitely implicated.

It is now well established that the histocompatibility antigen HLA B27 is closely associated with AS and the seronegative spondarthritides (Brewerton, 1976; Goldin and Bluestone, 1976). Reactive arthritis following infection with Salmonella, Shigella, and Yersinia enterocolitica also occurs predominantly in subjects carrying HLA B27 (Aho et al., 1975; Calin and Fries, 1975), and sacroiliitis and spondylitis are well recognised sequelae.

There is no satisfactory explanation of why the presence of HLA B27 predisposes to inflammatory joint and eye disease. The most favoured theory (McDevitt and Bodmer, 1974) proposes that a second gene closely linked to B27 is responsible for disease expression. Family studies suggest, and most investigators agree, that some other factor, possibly exogenous, is required to initiate the disease. We favour the molecular mimicry or cross-reactivity theory (Damian, 1964; Snell, 1968; Ebringer and Davies, 1973). Cross reactions between many different human tissue and bacterial antigens are well recognised (Jenkin, 1963; Dumonde, 1966).

The HLA molecule is a glycoprotein and therefore it is not inconceivable that there may be structural and antigenic similarities between HLA and bacterial cell components. Cross reactions between Gram-negative bacterial cell components and HLA antigens have now been reported (Hirata et al., 1973). Such a cross reactivity may result in an inadequate or delayed immune response by a B27-positive individual against infecting micro-organisms which carry similar antigens, because these organisms would be to some extent recognised as self.

We have observed cross reactivity between B27-positive lymphocytes and several Gram-negative bacteria: Enterobacter aerogenes, Klebsiella pneumoniae, and Yersinia enterocolitica (Ebringer et al., 1976). An initial clinical study suggested that Klebsiella pneumoniae was found more frequently in faeces of patients with active disease (Ebringer et al., 1975). We now report a larger series of patients, some of whom have been followed up for 18 months.

In view of the fluctuating course of the disease and longitudinal studies were carried out to determine if it was possible to establish a time relationship between the presence of Klebsiella and disease activity. Such a study might indicate the way in which the inflammatory disease was produced.

Patients and methods

163 patients have now entered the study. All except 18 fulfilled standard criteria for AS (Kellgren et al., 1963). These 18 patients had either Reiter’s syndrome.
or an incomplete or early AS with back pain and stiffness, peripheral lower limb joint involvement, and normal or equivocal sacroiliac joints on x-ray, and they all carried the B27 antigen. 4 other patients had spondylitis associated with inflammatory bowel disease and 3 had psoriatic spondylitis. 115 patients have been tissue typed and of these, 110 (96\%) carried the B27 antigen. 36 (22\%) of the patients were female.

157 control subjects were studied: staff, students, and a small number of ambulant, convalescent patients without arthritic disease. No patient or control included in this study was taking antibiotics at the time of assessment. The mean age and sex of the patients and controls are shown in Table 1.

Each patient provided a stool specimen passed within the previous 24 hours. Patients were then examined and assessed for clinical disease activity. They were usually seen at 3-monthly intervals unless the disease warranted more frequent attendance. They were to attend again at the earliest possible moment if symptoms became worse or they had a ‘flare-up’. There is a preponderance of younger patients in our survey due to our efforts to investigate the more active, early phase of the disease.

ASSESSMENT OF DISEASE ACTIVITY

The clinical assessment of patients into three classes of disease activity has been described (Ebringer et al., 1977). The following criteria were used in the assessment.

Active disease
Evidence of an active peripheral synovitis, effusion, or acute anterior uveitis. If signs and symptoms were related to spinal and joint pain and stiffness alone, then it was considered important that the onset, or exacerbation, was of recent origin (within the past 4 weeks). Increased dosage of anti-inflammatory drugs was usually noted.

Probably active disease
Persistent symptoms of pain or early morning stiffness requiring continuous anti-inflammatory therapy. Short fluctuations of disease symptoms were disregarded.

Table 1  Age and sex of patients and controls

<table>
<thead>
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<th>n</th>
<th>Male</th>
<th>Female</th>
<th>Mean age (years)</th>
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<tbody>
<tr>
<td>Patients</td>
<td>163</td>
<td>127 (78%)</td>
<td>36 (22%)</td>
<td>34.5</td>
</tr>
<tr>
<td>Controls</td>
<td>157</td>
<td>114 (73%)</td>
<td>43 (27%)</td>
<td>24.0</td>
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Inactive disease
Required little or no medication, and symptoms, when present, usually related more to the presence of chronic spondylitic changes in the joints or spine. A raised erythrocyte sedimentation rate alone did not preclude the assessment of inactive disease.

BACTERIOLOGY

Due to the small number of positive isolations obtained from midstream urine culture in our preliminary study, we did not repeat this investigation after the first visit. All faecal specimens were cultured and examined for the presence of Klebsiella/Enterobacter species, and Y. enterocolitica. Faecal specimens were cultured on a differential medium containing inositol, and incubated at 37°C for 18 hours before examination for inositol-fermenting colonies. Isolation of Y. enterocolitica was facilitated by incubation at 37°C for 48 hours preceded by enrichment culture using peptone water, pH 7.0 at 4°C for 3 weeks (Bejot et al., 1975). Pure isolates of suspect colonies were identified by the API 20E system.

STATISTICAL ANALYSIS

Our findings over 18 months were analysed in three different ways. In the latter two methods patients with active disease and probable active disease were combined and compared against patients with inactive disease.

Direct comparison of disease activity at each visit with faecal culture
Grading of clinical assessment of disease activity at each visit was correlated with the results of the faecal culture. Comparison between activity gradings and control groups were performed by $\chi^2$ analysis.

Comparison between disease activity and faecal cultures assessed over 3-monthly intervals
In the second method only one assessment of disease activity was included within a 3-month interval, even if the patient had attended on several occasions. The highest activity grading was recorded. Similarly, if only one culture was positive during those 3 months despite all other cultures being negative, then the patient was considered to have a positive faecal culture for that 3-month period. This method of grouping the observations into 3-monthly intervals was used to try to eliminate possible bias due to repeated sampling of select number of patients over a short period and to compensate for the finding of negative cultures during an active period when at least one culture during that interval was positive.
Comparison between positive and negative faecal cultures in patients with inactive disease and the subsequent disease activity.

We had noted that several occasions when an assessment of nonactive disease was made but the culture was positive the patient subsequently developed exacerbation of symptoms. To assess this statistically we noted all occasions at which patients were assessed as having nonactive disease and a follow-up assessment was available. 95 patients and 196 episodes were available for study. Analysis was based on the null hypothesis that a finding of a positive or negative culture in a nonactive patient should have no bearing on the subsequent assessment of disease activity. Patients were therefore assigned to four groups on the basis of their culture being positive or negative and whether they subsequently were assessed as having active or inactive disease. χ² analysis was performed on the expected and observed results.

Results

DIRECT COMPARISON OF DISEASE ACTIVITY AT EACH VISIT WITH FAEAL CULTURE

The 163 patients were seen on 433 occasions at which faecal specimens were available for analysis (Fig. 1). KL. pneumoniae was isolated on 178 occasions from positive cultures, E. aerogenes was isolated on only 9 occasions. No Y. enterocolitica micro-organisms were isolated. Positive cultures for Klebsiella/Enterobacter spp. were found on 33 (79%) of 42 occasions that active disease was assessed, on 66 (40%) of 164 occasions that probably active disease was assessed, and on 37 (16%) of 227 occasions that inactive disease was assessed. The rate of isolation of Klebsiella/Enterobacter spp. therefore appears to correlate with assessment of disease activity.

Statistical analysis (Table 2) showed that the difference in isolation rate between each of the disease activity groups was significant (P<0.001). The higher isolation rate in active disease and the lower isolation rate in inactive disease, compared to the control group was also significant (P<0.001). There was no difference between the control and the probably active disease group, or between the control and all the patient groups combined.

COMPARISON BETWEEN DISEASE ACTIVITY AND FAEAL CULTURES ASSESSED OVER 3-MONTHLY INTERVALS (FIG. 2)

For the purpose of analysis the assessments of active disease and probably active disease were combined into one composite active group. Of the 144 intervals assessed as active disease, 90 (63%) were found to have at least one positive culture for Klebsiella. In contrast, 29 (16%) of the intervals assessed as inactive disease were positive for Klebsiella. The difference between these groups is significant (χ²=78.2; P<0.001).

<table>
<thead>
<tr>
<th>Groups</th>
<th>χ²</th>
<th>Significance</th>
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<tr>
<td>Active vs Inactive</td>
<td>71.39</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Active vs Control</td>
<td>28.85</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Active vs Probably active</td>
<td>19.69</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Probably active vs Inactive</td>
<td>28.13</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Probably active vs Control</td>
<td>2.09</td>
<td>NS</td>
</tr>
<tr>
<td>Inactive vs Control</td>
<td>13.76</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>All patients vs Control</td>
<td>0.06</td>
<td>NS</td>
</tr>
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</table>

Fig. 1 Comparison between positive faecal cultures for Klebsiella/Enterobacter and individual disease activity assessment during 433 visits in 163 patients with ankylosing spondylitis.

Table 2. Comparison of disease activity groups, control subjects, and faecal cultures for Klebsiella/Enterobacter χ² analysis (assessments of individual visits and cultures).

Fig. 2 Comparison between disease activity and faecal cultures assessed over 3-monthly intervals. *Denotes combined active and probably active assessment groups.
COMPARISON BETWEEN POSITIVE AND NEGATIVE FAECAL CULTURES IN PATIENTS WITH INACTIVE DISEASE EPISODES AND THE SUBSEQUENT DISEASE ACTIVITY

For 95 patients with inactive disease episodes assessed on 196 occasions, a subsequent assessment of disease activity was available (Fig. 3). Patients were divided into four classes on the basis of positive or negative cultures for Klebsiella/Enterobacter and on whether subsequent assessment of disease activity had remained the same or worsened. Of the 161 episodes assessed as inactive with negative culture, 26 (16%) were subsequently assessed as having active or probably active disease (combined active group) on the next visit. Of the 35 episodes assessed as inactive with positive cultures, 15 (43%) developed clinical worsening (combined active group). The difference is significant ($\chi^2=12.4; P<0.001$).

CASE HISTORY

This case (Fig. 4) illustrates the course and pattern of disease frequently observed. A 26-year-old male student was first seen on 25 May 1976, giving a history of low back pain with early morning stiffness present for 10 years. Over the past few years symptoms had become milder and on first assessment he had few symptoms. Lumbar spinal flexion and chest expansion were limited and x-rays showed bilateral sacroiliitis. ESR was 3 mm/h. HLA typing was B27 positive. Visual analogue, self assessed, pain score was 2/20 and he was taking no anti-inflammatory drugs at that time. We assessed inactive disease. Faecal culture was negative for Klebsiella/Enterobacter spp.

On subsequent visits his clinical status did not change; however, a positive culture was obtained on 15 September. 4 weeks later a 'flare-up' of symptoms occurred with severe low back pain, early morning stiffness, and a short episode of diarrhoea. He was not seen at that time and upon return from holiday he presented on 9 November with continued severe symptoms, visual analogue pain, score 19/20. Probably active disease was assessed. Faecal culture was again positive for Klebsiella. He was started on phenylbutazone 200 mg daily with a good response. One week later pain score had decreased and faecal culture was negative. He has been seen twice since, his condition assessed as inactive, and faecal cultures negative.

Discussion

These results have confirmed our earlier observations that there is an association between Kl. pneumoniae and active disease in patients with AS. Klebsiella is an ubiquitous organism commonly found in the gastrointestinal tract. As yet no pathological significance has been attached to its presence there except for several reports that it may be a cause of neonatal enterocolitis (Reisner and Garty, 1977). Our studies now suggest that this micro-organism, in the gastrointestinal tract, may be a causal agent in the production of inflammatory arthritic disease in susceptible individuals. How the inflammatory disease may be produced is far from clear. If the B27-positive individual fails to respond adequately
then it is possible that the micro-organism may have some selective survival advantage. Endotoxin and other bacterial debris, or even bacteria, may be released into the circulation. The sites of predominant joint inflammation in AS are the sacroiliac joints and lumbar spine, and these are closely related to the lymphatic drainage of the pelvic floor and lower gastrointestinal tract.

Patients with AS and B27-positive subjects have been shown to have a diminished lymphocyte stimulation response to *Y. enterocolitica* (Nikbin et al., 1975), while the response to nonspecific stimulation with phytohaemagglutinin is normal (Nikbin et al., 1975; Fan et al., 1977). However, patients with reactive arthritis due to *Y. enterocolitica*, most of whom carry the B27 antigen, develop an adequate serological response to the organism with raised antibody titres. It is this paradox which confuses the whole issue. Similarly, Zilko et al. (1977) reported that patients with AS have raised antistreptolysin O titres despite a depressed skin response to streptococcal antigen. We therefore may be dealing with a disease condition in which is possible both a depressed immune response (due to cross reactivity) and a normal or even exaggerated immune response, possibly directed at structures in human tissues, which resemble bacterial antigens. The same paradox occurs in rheumatic fever. Patients who develop tonsillitis and rheumatic fever have higher and delayed antistreptolysin O titres than individuals who develop streptococcal tonsillitis alone (Winblad et al., 1949). Yet the rheumatic fever patients often develop repeated attacks of streptococcal infection and are more frequently carriers of streptococci. The mechanism of immune-response (IR) inversion (Young et al., 1978), may explain these phenomena.

There is little information on antibody levels to enteric bacteria in AS. Ford et al. (1977) found no evidence of raised titres to *Y. enterocolitica* in blood donors, patients with rheumatoid arthritis, Haida Indians, patients with AS, or patients with Reiter's syndrome. However, 4 patients out of 28 with acute arthritis did have serological evidence of recent *Yersinia* infection. 3 of these patients had the B27 antigen. Raised antibody titres to enteric bacteria have been reported in children with juvenile chronic polyarthritis (Gutowska-Grzegorzuk and Baum, 1977), but no analysis on the basis of B27 positivity was reported.

Our results have also shown a significantly lower incidence of *Klebsiella* in the faeces of patients with inactive disease when compared to controls. There may be several explanations for this. The incidence of positive cultures reported for our control group may be higher than the incidence in the general population. Samples were in the main collected from healthy hospital volunteers. Nearly all our patients were outpatients. The incidence of positive cultures for *Klebsiella* increases on contact with a hospital environment (Seldon et al., 1971). Another possibility is that patients with inactive disease in some ways have acquired 'immunity' against infections or persistence of the micro-organisms. This remains to be tested.

Our observations of patients with AS over an extended period has enabled us to assess the temporal relationship between positive cultures for *Klebsiella* and active disease. It is apparent (see case history) that although there is an association of *Klebsiella* with active disease, the two events are slightly out of phase with each other. *Klebsiella* often appears before the inflammatory disease and disappears before the inflammation subsides. In this respect it is analogous to the situation in acute rheumatic fever where the streptococcal infection appears first and rheumatic fever appears later although the streptococcus can often still be isolated from the throat in the acute phase. For this reason we attempted to assess the results in 3-monthly intervals, hoping that this form of assessment would overcome the problem of the lag period and also the slower decrease of the inflammatory disease activity. The results again show the association between active disease and at least one positive faecal culture during the 3-monthly intervals.

Sequential analysis confirmed our clinical impression that patients with inactive disease and positive cultures often went on to develop a subsequent flare-up of the disease.

These findings still do not enable us to propose a definite causal effect of *Klebsiella* infection on disease activity in AS. However, the finding that *Moraxella pneumonieae* appears before exacerbation of the disease strengthens the hypothesis that subclinical infection by *Klebsiella* in the gastrointestinal or genitourinary tract leads to clinical manifestations of inflammatory joint disease.

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