

Frequency of faecal *Klebsiella aerogenes* in patients with ankylosing spondylitis and controls with respect to individual features of the disease

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SUMMARY A study of 59 patients with definite ankylosing spondylitis and 41 comparable hospital outpatients with fractures has been undertaken to determine if the presence of faecal *Klebsiella aerogenes* is related to clinical activity of the spinal disease and its extraspinal features. The frequencies of faecal *K. aerogenes* were similar in both patients and controls and were not significantly related to spinal disease activity. Careful inquiry about antibiotic treatment, dietary habits, and hospitalisation did not significantly influence the results. A significant association was found between the presence of faecal *K. aerogenes* and both acute non-granulomatous anterior uveitis ($P < 0.01$) and peripheral synovitis in HLA B27 positive patients ($P < 0.05$). These results suggest that *K. aerogenes* may have an aetiological role in the development of non-granulomatous anterior uveitis and peripheral arthritis in patients with ankylosing spondylitis but do not lend support to this organism having such a role in the spinal disease itself.

Although genetic factors are important in the aetiology of ankylosing spondylitis (Emery and Lawrence, 1967; Brewerton *et al.*, 1973; Schlosstein *et al.*, 1973) the study of identical twins suggests that additional environmental factors are probably necessary for the development of this disease (Moesmann, 1960; Eastmond and Woodrow, 1977). On the basis of their findings of cross-reactivity and antigenic similarity between *Klebsiella aerogenes* and HLA B27 positive lymphocytes (Young *et al.*, 1978) Ebringer *et al.* (1977, 1978) have studied the occurrence of this organism in patients with ankylosing spondylitis. They found *K. aerogenes* in faeces of patients with active disease more frequently than in those with inactive disease and controls, and suggested that this organism may be an important exogenous agent causing the disease and its exacerbations.

The present study has been performed in order to determine if these results can be reproduced and

whether faecal *Klebsiella aerogenes* is associated with any particular features of the disease.

Patients and controls

Fifty-six outpatients with definite ankylosing spondylitis alone (Bennett and Wood, 1968) were asked to supply a faecal specimen to be returned to us by post. A standard proforma was used to record the details of their disease and current medication together with the place and type of regularly taken meals. Antibiotics, infections, and periods of hospitalisation in the preceding 6 months were also recorded, since these may affect faecal carriage of *Klebsiella aerogenes* (Eickhoff, 1970). An assessment of current spinal disease activity was made on the basis of the duration of early morning stiffness and the dose of nonsteroidal anti-inflammatory drugs required to control symptoms.

Three categories of disease activity were defined at the start of the study. (1) *Inactive*. Patients with both (a) less than 15 minutes' early morning stiffness and (b) requiring less than either 50 mg of indomethacin, 100 mg of phenylbutazone, or an

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equivalent dose of an alternative drug per 24 hours for control of symptoms. (2) *Definitely active*. Patients with both (a) 1 hour or more of early morning stiffness and (b) requiring more than 100 mg of indomethacin, 200 mg of phenylbutazone, or an equivalent dose of an alternative drug per 24 hours for control of symptoms. (3) *Probably active*. Patients who failed to satisfy the criteria for either of the above categories of disease activity.

The presence and site of peripheral synovitis was noted, as was the presence of acute anterior uveitis. This latter diagnosis was confirmed by an ophthalmologist when necessary.

In addition 13 patients known to have ankylosing spondylitis and presenting with acute non-granulomatous anterior uveitis at ophthalmology clinics were documented and studied in an identical manner.

An anteroposterior radiograph of the pelvis and both anteroposterior and lateral radiographs of the lumbar spine were obtained in all patients with ankylosing spondylitis and read subsequently by 2 observers (CJE and VW) without knowledge of the clinical or bacteriological details of the individual patients. Sacroiliitis was graded according to the New York criteria (Bennett and Wood, 1968). The presence and number of Romanus lesions (Romanus and Yden, 1955), squared vertebrae, and syndesmophytes were noted in the lumbar spine. On the basis of these readings the patients were considered to have radiologically mild or moderate radiological disease if they had grade 3 or less radiological sacroiliitis with only 1 syndesmophyte without evidence of posterior spinal fusion, and severe radiological disease if 2 or more syndesmophytes or posterior spinal fusion were present irrespective of the grade of radiological sacroiliitis.

Sixty-six controls of similar age and sex to the patients were seen at routine fracture clinics of orthopaedic departments and requested to supply a faecal specimen, using the same method as the patients. The same proforma was used to record the details of the place and type of regularly taken meals, infections, the use of antibiotics, and any spells in hospital in the preceding 6 months.

METHOD FOR COLLECTION OF FAECES

These were collected by the patient in his own house. After micturition a large polyethylene bag was laid over the water closet seat and faeces passed into the bag. With a sterile plastic spoon a sample was taken and transferred to a sterile container, which was then returned by post to our laboratory.

LABORATORY ISOLATION AND CULTURE

Klebsiella aerogenes was isolated from faeces by

means of selective and enrichment media, identified, and serotyped as previously reported (Cooke *et al.*, 1979).

Results

Forty-eight (85.7%) of the patients (41 male) with ankylosing spondylitis alone and 41 (62.1%) of the controls (36 male) returned faecal specimens for bacteriological examination. The mean age of the patients was 39.4 years (range 18–64 years) and of the controls was 36.0 years (range 17–64). Histo-compatibility typing was available for 34 of the patients, of whom 29 (85.3%) were HLA B27 positive. All 13 additional patients with acute non-granulomatous anterior uveitis returned faecal specimens.

Klebsiella aerogenes was detected in the faeces of 13 (27.1%) of the patients with ankylosing spondylitis alone compared with 14 (34.1%) of controls. A similar proportion (27.6%) of HLA B27 positive patients were found to have faecal *K. aerogenes* as in the total patient group (27.1%) (Table 1). No significant differences were apparent.

Klebsiella aerogenes was recovered from the faeces more frequently in patients with definitely active disease than from those with inactive disease and controls, but the differences failed to reach statistical significance (Table 2). In both the HLA B27 positive patients and the total group *K. aerogenes* was recovered from the faeces more frequently in patients with definitely active disease than from those with inactive disease and controls, but the differences failed to reach statistical significance (Table 2).

Klebsiella aerogenes was recovered more frequently from the faeces of patients who assessed their pain as moderate or severe than from those with mild or no pain (Table 3), but the differences failed to reach statistical significance.

Klebsiella aerogenes was recovered from the faeces of the 13 patients with acute anterior uveitis significantly more frequently than from the 48 patients

Table 1 Recovery of *Klebsiella aerogenes* from the faeces of patients with ankylosing spondylitis with respect to the presence of HLA B27 and from controls

Diagnostic group	HLA B27	Patients with faecal <i>Klebsiella aerogenes</i>		Total
		Number	Percent	
Patients with ankylosing spondylitis	Positive	8	27.6	29
	Negative	3	60.0	5
	Not known	2	14.3	14
	All	13	27.1	48
Controls		14	34.1	41

Table 2 Recovery of *Klebsiella aerogenes* from the faeces of patients with ankylosing spondylitis with respect to the presence of HLA B27 and spinal disease activity and in controls

Disease activity	HLA B27 present		HLA B27 absent		HLA B27 not known		All patients	
	Total number	Number with faecal <i>Klebsiella aerogenes</i>	Total number	Number with faecal <i>Klebsiella aerogenes</i>	Total number	Number faecal <i>Klebsiella aerogenes</i>	Total number	Number with faecal <i>Klebsiella aerogenes</i>
Inactive	7	2 (28.6%)	0	0	4	0	11	2 (18.2%)
Probably active	16	3 (18.8%)	2	2 (100.0%)	7	1 (14.3%)	25	6 (24.0%)
Definitely active	6	3 (50.0%)	3	1 (33.3%)	3	1 (33.3%)	12	5 (41.7%)
Controls	—	—	—	—	—	—	41	14 (34.1%)

Table 3 Recovery of *Klebsiella aerogenes* from the faeces of patients with ankylosing spondylitis with respect to spinal pain severity

Diagnostic group	Pain severity	Patients with faecal <i>Klebsiella aerogenes</i>		Total	
		Number	Per cent		
Patients with ankylosing spondylitis	HLA B27 positive	Nil	1	25.0	4
		Mild	1	10.0	10
		Moderate	4	36.4	11
		Severe	2	50.0	4
All		Nil	1	20.0	5
		Mild	2	11.1	18
		Moderate	8	42.1	19
		Severe	2	33.3	6
Controls			14	34.1	41

without uveitis (corrected $\chi^2=11.92$ (1 DF); $P<0.001$) and from the 41 controls (corrected $\chi^2=8.18$ (1 DF); $P<0.01$) (Table 4). The clinical activity of the spinal disease in patients with uveitis on the same criteria as for patients without uveitis did not relate to the presence of *K. aerogenes*. Only 3 had definitely active disease and 4 had clinically inactive spinal disease. Two patients had been seen and had supplied faecal samples before the development of their uveitis, when *K. aerogenes* could not be recovered from these specimens. One of these patients had had a coincidental increase in clinical activity of the spinal disease from inactive to definitely active, whereas the other patient had experienced no change in his spinal symptoms.

Table 4 Recovery of *Klebsiella aerogenes* from the faeces of patients with ankylosing spondylitis with respect to the presence or absence of acute non-granulomatous anterior uveitis and from controls

Patients	Patients with faecal <i>Klebsiella aerogenes</i>		Total number of patients
	Number	Per cent	
Acute uveitis + ankylosing spondylitis	11	84.6	13
Ankylosing spondylitis alone	13	27.1	48
Controls	14	34.1	41

Twenty-three patients had an active synovitis of at least 1 peripheral joint and no uveitis. *Klebsiella aerogenes* was recovered from the faeces twice as frequently in these 23 patients as in the 25 patients without peripheral arthritis and uveitis (corrected $\chi^2=2.18$ (1 DF); $0.2>P>0.1$) (Table 5). Fifteen HLA B27 positive patients had an active peripheral joint synovitis, and in 7 of these (46.7%) *K. aerogenes* was recovered from the faeces compared with 1 (7.1%) of the 14 HLA B27 positive patients lacking a peripheral arthritis (corrected $\chi^2=3.86$; $P<0.05$) (Table 5).

The mean duration of disease in the 13 patients with faecal *Klebsiella aerogenes* was 16.3 years (range 5–32 years) and in those without faecal *K. aerogenes* 15.1 years (range 1–38 years). Radiographs were available for 47 patients. Three of the 13 patients with radiologically mild or moderate disease were found to have faecal *K. aerogenes* compared with 9 of the remaining 34 patients with severe radiological disease.

Ten patients and 10 controls had received antibiotics in the preceding 6 months. Three of these patients and 5 of these controls had *Klebsiella aerogenes* recovered from their faeces compared with 10 and 9 of the remaining 38 patients and 31 controls respectively who had not received antibiotics.

Eight of the 16 controls who ate their midday meal in a canteen or restaurant had *Klebsiella aerogenes*

Table 5 Recovery of *Klebsiella aerogenes* from the faeces of patients with ankylosing spondylitis related to the presence of peripheral synovitis

HLA B27	No. of patients with peripheral synovitis	Patients with faecal <i>Klebsiella aerogenes</i>				Total number of patients
		Peripheral synovitis present		Peripheral synovitis absent		
		Number	Per cent	Number	Per cent	
Positive	15	7	46.7	1	7.1	29
Negative	2	1	50.0	1	66.6	5
Not known	6	1	16.6	1	12.5	14
All	23	9	39.1	3	16.0	48

recovered from their faeces compared with 6 of the remaining 25 controls. Only 4 of these had a cooked meal, of whom 3 had *K. aerogenes* in their faeces. Fifteen patients had canteen or restaurant midday meals and 4 of these had *K. aerogenes* in their faeces compared with 9 of the remaining 33 patients.

Fourteen controls had been hospitalised for periods up to 3 weeks in the preceding 6 months. *Klebsiella aerogenes* was recovered from the faeces of 6 of these controls compared with 8 of the 77 who had not been hospitalised. Eight patients had been similarly hospitalised for up to 4 weeks and one for 7 weeks. Three of these 9 patients were found to have *K. aerogenes* in their faeces compared with 10 of the 39 patients not hospitalised.

Discussion

The definition of active ankylosing spondylitis used by Ebringer *et al.* (1977, 1978) was based on the presence of 1 of 3 features: (1) a recent worsening of spinal symptoms, (2) the presence of acute anterior uveitis, and (3) the presence of an active peripheral synovitis. In the present study these last 2 features have been excluded from our definition of disease activity and have been analysed separately. Acute anterior uveitis may occur in the absence of ankylosing spondylitis in HLA B27 positive individuals (Brewerton, 1975; Mapstone and Woodrow, 1975). In addition Eastmond and Woodrow (1977) noted discordance for acute anterior uveitis in a pair of identical twins, the twin with classical ankylosing spondylitis never having uveitis, whereas his identical twin who did not have classical ankylosing spondylitis had had uveitis. These studies suggest that, although acute anterior uveitis may occur in 25% of patients with ankylosing spondylitis (Wilkinson and Bywaters, 1958), this is probably because of common genetic factors predisposing to the 2 disorders rather than common environmental factors.

A peripheral synovitis occurs in approximately one-quarter of patients with ankylosing spondylitis (Wilkinson and Bywaters, 1958). It may, however, precede the onset of spinal symptoms by several years (Hart and MacLagan, 1955; Wilkinson and Bywaters, 1958; Ladd *et al.*, 1971; Riley *et al.*, 1971). Several groups have noted the occurrence of such an arthritis in HLA B27 positive individuals who do not have spinal disease (Keat and Barnes, 1976; Arnett *et al.*, 1976). Only prolonged follow-up will determine what proportion of these patients ultimately develop ankylosing spondylitis. The gastrointestinal symptoms of reactive arthritis (Berglof, 1963) may be mild and their importance not recognised by the patient. The absence of any

other features of Reiter's syndrome could make precise diagnosis difficult. As with acute anterior uveitis, it is possible that the common factor between ankylosing spondylitis and its associated peripheral arthritis is genetic rather than environmental (Aho *et al.*, 1975).

Unfortunately the clinical activity of the spinal features of ankylosing spondylitis are difficult to determine. Even minor physical trauma can be followed by an exacerbation of pain. The patient may use drugs to control his symptoms, but there is no evidence to suggest that they alter the underlying disease process. Radiotherapy may temporarily abolish symptoms, but radiological evidence of progression may still occur (Wilkinson and Bywaters, 1958). For these reasons we have chosen to define spinal disease activity on the basis of 2 measurable variables, the duration of early morning spinal stiffness and the dose of anti-inflammatory drug used to control symptoms. Using our definition of inactive and definitely active disease we have been able to separate those patients with trivial symptoms from those with continuing severe, uncontrolled symptoms respectively. The remaining patients have various degrees of disease activity between these 2 extremes.

The results of the present study, while suggesting that patients with severe active ankylosing spondylitis may have faecal *Klebsiella aerogenes* more frequently than those with inactive disease and controls, must be interpreted with caution in view of their lack of statistical significance. It is noteworthy that Warren and Brewerton (1978), using a similar definition of spinal disease activity as Ebringer *et al.* (1977, 1978) have failed to find any association between increase in symptoms and the aecal carriage of *K. aerogenes*.

Our finding of *Klebsiella aerogenes* in 84.6% of patients with active acute anterior uveitis is of special interest as the presence of this feature was of cardinal importance in the definition of active disease used by Ebringer *et al.* (1977, 1978). This and the association of *K. aerogenes* with peripheral synovitis probably explain the apparent discrepancies between the 2 studies. The significant association between the presence of active peripheral synovitis and faecal *K. aerogenes* in HLA B27 positive patients suggests the possibility of a causal relationship between *K. aerogenes* and peripheral arthritis in HLA B27 individuals.

The dietary habits, antibiotic usage, and hospitalisation in the preceding 6 months were comparable in patients and controls with similar effects of the frequency of faecal *Klebsiella aerogenes* in both groups. It is unlikely therefore, that these factors will have significantly affected the comparison of

faecal carriage rates of this organism between patients and controls.

If the pathogenicity of *Klebsiella aerogenes* is related to the persistence of antigen and the subsequent development of cross-reacting antibodies with HLA B27 due to cross-tolerance, HLA B27 negative patients would be expected to have faecal *K. aerogenes* with a similar frequency as controls and less frequently than HLA B27 positive patients. In the present study 5 patients were known to be HLA B27 negative, of whom 3 (60%) had faecal *K. aerogenes* compared with 34.1% of 41 controls. One of 3 HLA B27 negative patients with definitely active disease and both such patients with probably active disease had faecal *K. aerogenes*. HLA B27 negative patients with ankylosing spondylitis are uncommon, but the frequency of faecal *K. aerogenes* in these 5 patients does not suggest that they differ from the 29 HLA B27 positive patients with respect to faecal *K. aerogenes*. In addition they appear overall to have faecal *K. aerogenes* more frequently than the controls.

Environmental factors related to the development of a disease might be expected to be associated with the early rather than the late stages of the disease. The finding of no differences in the mean duration of clinical ankylosing spondylitis in those patients with and without faecal *Klebsiella aerogenes* and the finding of *K. aerogenes* with equal frequency in patients with radiologically mild or moderate disease compared with those with radiologically severe disease is additional evidence against the pathogenic role of this organism in the spinal disease.

Conclusions

The results of the present study suggest that in patients with ankylosing spondylitis: (1) The development of acute non-granulomatous anterior uveitis appears to be related to the presence of *Klebsiella aerogenes* in the gastrointestinal tract. (2) The development of peripheral synovitis may be related to the presence of *K. aerogenes* in the gastrointestinal tract in HLA B27 positive individuals. (3) The presence of gastrointestinal *K. aerogenes* is not a constant feature in patients with clinical activity of their spinal disease and is not related to the degree of pain, duration of the disease, or severity of x-ray changes or the presence of absence of HLA B27 (except when there is a peripheral synovitis).

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