

## EXTENDED REPORTS

## Systemic and mucosal antibodies to klebsiella in patients with ankylosing spondylitis and Crohn's disease

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### Abstract

Whole gut lavage fluid is a useful source of material for the study of intestinal immunity and inflammation in humans. Systemic and mucosal antibodies to *Klebsiella pneumoniae* were measured by enzyme linked immunosorbent assay (ELISA) in serum samples and whole gut lavage fluid from 14 patients with ankylosing spondylitis, 14 with Crohn's disease, and 16 immunologically normal controls. As the concentration of IgG in whole gut lavage fluid reflects disease activity in Crohn's disease, this approach was used to detect intestinal inflammation in patients with ankylosing spondylitis who also had disease activity and use of non-steroidal anti-inflammatory drugs (NSAIDs) recorded. Small intestinal permeability to cellobiose and mannitol was also studied. In serum samples, levels of IgA antibody to klebsiella were high in patients with Crohn's disease and in patients with active ankylosing spondylitis, and were significantly correlated with the erythrocyte sedimentation rate in patients with ankylosing spondylitis. Levels of IgG antibody to klebsiella were also high in patients with Crohn's disease. Studies of whole gut lavage fluid showed similar levels of IgA antibody to klebsiella in the three study groups, but levels of whole gut lavage fluid IgM and IgG antibodies to klebsiella were high in patients with Crohn's disease. Levels of IgG in whole gut lavage fluid were high in patients with Crohn's disease but in only one patient with ankylosing spondylitis, though the cellobiose/mannitol permeability ratio was abnormal in eight of 13 patients with ankylosing spondylitis. It is concluded that high levels of serum IgA antibody to klebsiella are not specific to ankylosing spondylitis, and that there is no evidence of an abnormal intestinal IgA antibody response to klebsiella in patients with ankylosing spondylitis.

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The putative association between ankylosing spondylitis and intestinal infection with *Klebsiella pneumoniae*, and the possible relevance of possession of the HLA-B27 antigen, have stimulated much interest.<sup>1-4</sup> Ebringer and coworkers found faecal cultures positive for klebsiella in patients with ankylosing spondylitis with active disease,<sup>5,6</sup> but others found no correlation between faecal carriage of klebsiella

and disease activity.<sup>7,8</sup> Ebringer<sup>9</sup> postulated that antibody to klebsiella produced in the gut lymphoid tissues binds to HLA-B27 positive cells, with activation of the complement cascade; repeated episodes of 'klebsiella reactive arthritis' eventually produce the clinical syndrome of ankylosing spondylitis.

Studies of antibodies to klebsiella have given conflicting results, however. Trull and coworkers<sup>10,11</sup> reported high levels of serum IgA antibodies to klebsiella but not to other enteric organisms, whereas Van Bohemen *et al*<sup>12</sup> reported high titres of serum antibodies to klebsiella and also to other Enterobacteriaceae, and Pease *et al*<sup>13</sup> reported that titres of IgA antibody to klebsiella in patients with ankylosing spondylitis were normal. Cooper *et al*<sup>14</sup> found high levels of serum IgA antibody to klebsiella in patients with ankylosing spondylitis, but also in patients with rheumatoid arthritis and inflammatory bowel disease, and suggested that abnormal intestinal permeability<sup>15</sup> might explain the findings. This could be due to the effects of non-steroidal anti-inflammatory drugs (NSAIDs),<sup>16</sup> or might reflect intrinsic small intestinal inflammation in ankylosing spondylitis.<sup>17</sup>

To date, the intestinal humoral immune response to klebsiella in ankylosing spondylitis has not been studied. We have described a technique of whole gut lavage,<sup>18</sup> which is a non-invasive and simple means of collecting intestinal secretions for studies of humoral immunity. Using this technique we have characterised the secretory immune response to food antigens in coeliac disease.<sup>19</sup> Separately, we have also established that the concentration of IgG in gut lavage fluid is a sensitive marker of disease activity in inflammatory bowel disease.<sup>20</sup>

In this study we characterised the systemic and intestinal humoral immune responses to klebsiella in patients with ankylosing spondylitis, Crohn's disease, and in control subjects. We also determined the occurrence of altered intestinal permeability in patients with ankylosing spondylitis, and, by using a novel approach based on the assay of whole gut lavage fluid IgG, we investigated intestinal inflammation in patients with ankylosing spondylitis. A group of patients with Crohn's disease were studied in parallel because Cooper *et al* have reported high levels of serum IgA antibody to klebsiella in patients with inflammatory bowel disease, and because 'Crohn's-like' lesions have been reported to occur in most patients with ankylosing spondylitis.<sup>17</sup>

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## Subjects and methods

### PATIENTS

We studied 14 patients with ankylosing spondylitis; all patients fulfilled the New York diagnostic criteria.<sup>21</sup> They were divided into three categories of disease activity:<sup>22</sup> patients with 'active' disease had a high erythrocyte sedimentation rate (ESR) (>15 mm/hour) and high serum IgA (>3 g/l); patients with 'probably active' disease had either a high ESR or IgA concentration; and in patients with 'inactive' disease these two values were normal. We studied 14 patients with active Crohn's disease, seven men and seven women, median age 45, range 20–66 years. None had any form of arthritis, all had classical histological or radiological features, or both, and, on global assessment, were judged to have active disease. Sixteen control subjects were studied, five men and 11 women, median age 30, range 14–75 years. These were either normal volunteers, or patients with functional (that is, non-organic) bowel disease.

### GUT LAVAGE

Gut lavage was carried out as described previously.<sup>18</sup> Patients consumed up to 4 litres of Golytely, a polyethylene glycol based isotonic electrolyte lavage solution; this solution is widely used as a bowel preparation for barium enema, colonoscopy, and colonic surgery. Patients were asked to drink the lavage solution at a rate of 250 ml every 15 minutes. Stool collection began once the liquid passed through the rectum was clear and free of faecal material. Specimens were centrifuged and treated with protease inhibitors,<sup>18</sup> and stored at  $-70^{\circ}\text{C}$  until assayed.

### INTESTINAL PERMEABILITY TEST

Intestinal permeability was assessed by measuring the urinary excretion of two sugars, cellobiose and mannitol, and expressing the percentage recovered in urine as a cellobiose/mannitol (C/M) ratio.<sup>23</sup> The normal C/M ratio is  $<0.037$ .

### ENZYME LINKED IMMUNOSORBENT ASSAYS

#### *Antibodies to klebsiella*

The method used is essentially that described in

detail by Cooper *et al.*<sup>14</sup> *Klebsiella pneumoniae* K43 (National Collection of Type Cultures 9163) was used as the antigen. Samples were diluted as follows: serum, 1/500 (IgG and IgM) and 1/2000 (IgA); and gut lavage fluid, 1/4 (IgA, IgM, IgG). These sample dilutions gave optimum absorbances. A 100  $\mu\text{l}$  aliquot of the sample was added in triplicate to the wells. Control uncoated wells containing the sample alone were included for each sample. Results were obtained by taking the median absorbance for each sample. The specificity of the assay was established by Cooper *et al.*<sup>14</sup>

#### *Immunoglobulins*

Concentrations of IgA, IgM, and IgG in gut lavage fluid were measured by enzyme linked immunosorbent assay (ELISA) as described elsewhere.<sup>18</sup> Serum immunoglobulins were measured by an immunoturbidimetric method using an autoanalyser.

### STATISTICS

Differences in antibody and immunoglobulin levels between the three groups were analysed with the Mann-Whitney U test. Correlation coefficients were calculated using Spearman's rank correlation test.

## Results

### INTESTINAL PERMEABILITY AND FAECAL CULTURES IN PATIENTS WITH ANKYLOSING SPONDYLITIS

Table 1 gives the clinical details of the 14 patients with ankylosing spondylitis, including age, sex, ESR, serum IgA, C/M permeability ratio, and intake of NSAIDs. The C/M ratio was abnormal in eight of 13 patients tested. Also of note is the fact that the permeability ratio was abnormal in two of four patients not receiving NSAIDs at the time of study. Faecal culture for klebsiella was negative in all patients. The C/M ratio did not correlate with the ESR or the serum IgA level.

### GUT LAVAGE FLUID AND SERUM IMMUNOGLOBULIN

#### *Serum immunoglobulins*

Levels of serum IgA were significantly higher than controls in the patients with ankylosing spondylitis ( $p<0.05$  *v* controls) and the patients with Crohn's disease ( $p<0.05$  *v* controls) (table 2).

#### *Gut lavage fluid immunoglobulins*

In control patients, the most abundant whole gut lavage fluid immunoglobulin was IgA, with lesser amounts of IgM, and low ( $<10$  mg/l) IgG. As previously reported,<sup>18</sup> patients with active Crohn's disease had high levels of gut lavage fluid IgG ( $p<0.00001$  *v* controls) and IgM ( $p<0.001$  *v* controls); levels of whole gut lavage fluid IgA were also high ( $p<0.05$  *v* controls). Lavage fluid immunoglobulins in the patients with ankylosing spondylitis were similar to control subjects; only one patient with ankylosing spondylitis had an abnormally high

Table 1 Clinical details of patients with ankylosing spondylitis. Normal cellobiose/mannitol (C/M) ratio  $<0.037$

Patient No	Age (years)	Sex	ESR*	NSAIDs*	C/M ratio	Serum IgA (g/l)
Inactive disease						
1	27	M	9	Yes	—	2.37
2	40	M	3	Yes	0.040	2.16
3	28	M	8	No	0.017	1.61
4	53	F	10	Yes	0.080	1.12
5	24	M	—	Yes	0.029	2.64
'Probably active' disease						
6	35	M	28	No	0.034	2.30
7	50	M	11	No	0.092	3.23
8	50	M	27	Yes	0.051	2.67
9	59	F	27	Yes	0.137	1.67
10	32	M	10	No	0.082	4.01
'Active' disease						
11	46	F	17	Yes	0.023	4.92
12	61	M	22	Yes	0.081	4.50
13	41	F	34	Yes	0.056	5.31
14	24	M	57	Yes	0.010	4.17

\*ESR=erythrocyte sedimentation rate; NSAIDs=non-steroidal anti-inflammatory drugs.

Table 2 Serum (g/l) and whole gut lavage fluid (WGLF) ( $\mu$ g/ml) immunoglobulins (median and range)

Antibody	Control subjects	Patients with ankylosing spondylitis	Patients with Crohn's disease
Serum			
IgA	1.74 (1.03-5.53)	2.65* (1.12-5.31)	2.62* (1.83-3.40)
IgM	1.24 (0.37-3.82)	1.02 (0.50-2.47)	1.27 (0.16-2.14)
IgG	10.18 (5.39-14.96)	10.96 (7.48-19.14)	9.74 (5.66-20.96)
WGLF			
IgA	114 (11-535)	90 (28-298)	192* (74-1256)
IgM	7 (2-28)	6 (2-30)	17* (8-53)
IgG	0.9 (0.2-3.2)	2.0 (0.34-6)	39.5* (10.2-596.5)

\*Immunoglobulin levels significantly higher ( $p < 0.05$ ) than controls.

IgG level in whole gut lavage fluid. Serum and whole gut lavage fluid immunoglobulin levels are given in table 2.

#### ANTIBODIES TO *KLEBSIELLA PNEUMONIAE*

##### Serum antibodies

Control subjects had detectable antibodies to all three isotypes (fig 1). High levels of serum IgA antibody to klebsiella were found in the patients with active ankylosing spondylitis ( $p < 0.05$  v controls), but IgA antibody levels in the ankylosing spondylitis group as a whole were not significantly higher than in controls. There was

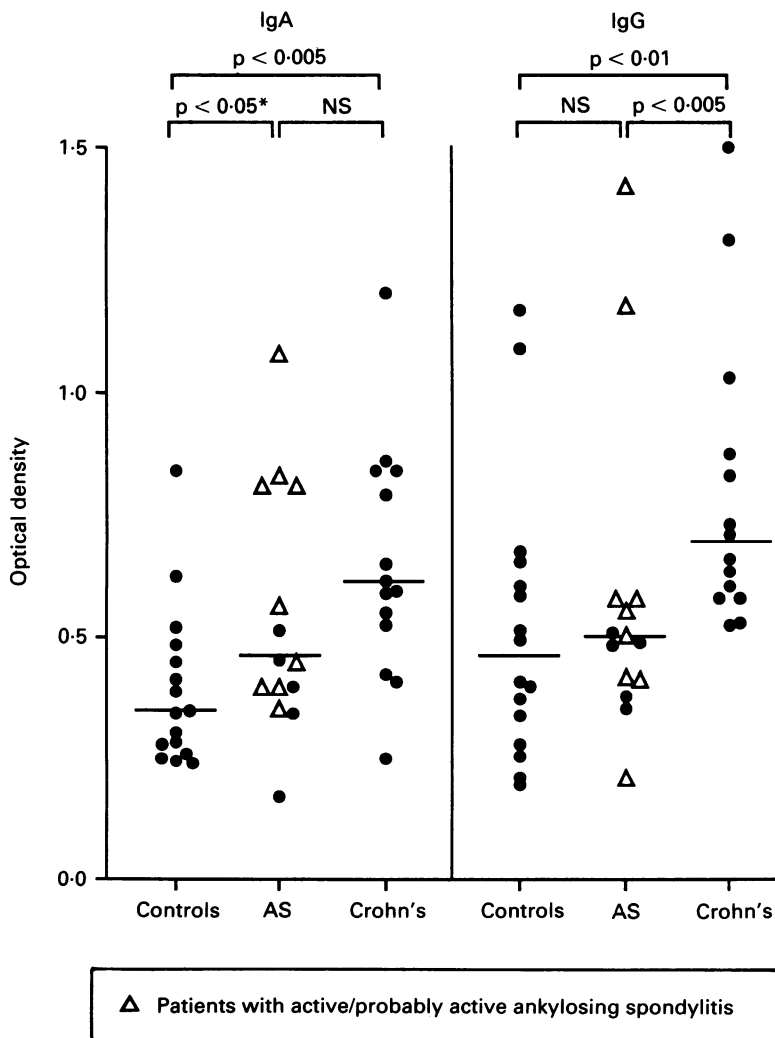


Figure 1 Serum levels of IgA and IgG antibodies to klebsiella. NS=not significant; AS=patients with ankylosing spondylitis. \*Antibody levels significantly higher only in patients with AS with active/probably active disease ( $\Delta$ ).

a significant correlation in these 14 patients between serum IgA levels of antibody to klebsiella and the ESR ( $r=0.72$ ;  $p < 0.005$ ) and the total serum IgA ( $r=0.77$ ;  $p < 0.002$ ); there was no significant correlation with the C/M ratio. In the patients with Crohn's disease, high levels of IgA and IgG antibodies to klebsiella were detected ( $p < 0.005$  and  $p < 0.01$  respectively v controls). Serum IgM antibody levels were similar in the three groups.

##### Gut lavage fluid antibodies

Most control subjects had detectable IgA antibody levels, and some also had antibodies of the IgM isotype (fig 2). Antibody levels in the ankylosing spondylitis group (in the group as a whole and in the patients with active disease) were similar to controls for all three isotypes. High levels of intestinal IgM and IgG antibodies to klebsiella were detected in the Crohn's disease group ( $p < 0.01$  and  $p < 0.0002$  v controls respectively). There was a trend towards high levels of whole gut lavage fluid IgA antibody to klebsiella in the patients with Crohn's disease, but this did not reach statistical significance. Whole gut lavage fluid levels of IgG antibodies to klebsiella in this group did not correlate with either total whole gut lavage fluid IgG, or serum levels of IgG antibody to klebsiella.

##### Discussion

We have shown that patients with active ankylosing spondylitis have high levels of serum IgA antibody to klebsiella, but this is not specific, as high antibody levels are also found in patients with Crohn's disease. More importantly, we have found no evidence of an abnormal mucosal antibody response to klebsiella in patients with ankylosing spondylitis. Conversely, patients with Crohn's disease have high titres of systemic and mucosal antibodies to klebsiella. It is likely that in patients with inflammatory bowel disease, intestinal bacterial antigens cross the inflamed mucosa, gaining access to the lamina propria where an antibody response is generated. High levels of serum IgA antibody in this group may be the result of spillage of intestinal antibody across the inflamed mucosa. Levels of whole gut lavage fluid IgG antibody to klebsiella were particularly increased in the patients with Crohn's disease; the lack of correlation between intestinal and serum antibody levels suggests that these antibodies may not be due to simple plasma leakage, but further studies are needed to establish the relative contribution of plasma leakage to whole gut lavage fluid immunoglobulins and specific antibodies.

We have not examined the systemic and mucosal humoral immune response to other Enterobacteriaceae. We predict that high intestinal antibody levels in patients with Crohn's disease are not confined to *Klebsiella pneumoniae*, as patients with Crohn's disease and ulcerative colitis have increased serum antibody titres to a wide range of bacteria, including klebsiella, *Escherichia coli*, bacteroides, and yersinia.<sup>24-26</sup> Furthermore, it has been

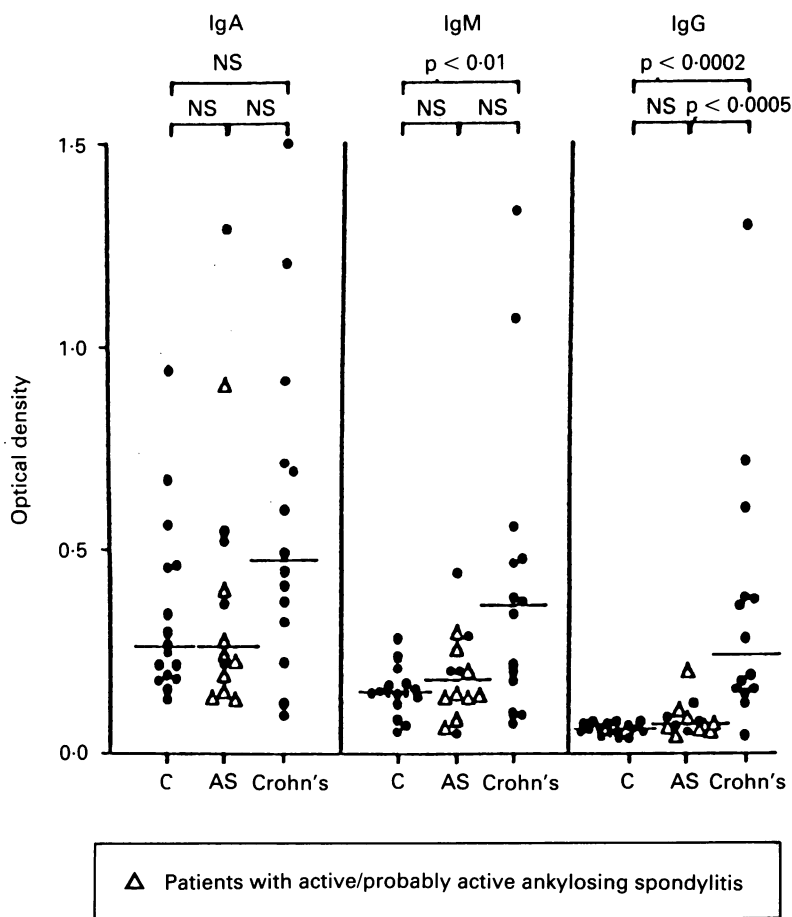


Figure 2 Levels of antibodies to klebsiella in whole gut lavage fluid. NS=not significant; C=controls; AS=patients with ankylosing spondylitis.

shown that considerable antigenic cross reactivity exists between klebsiella and other Gram negative enterobacteria, such as yersinia, pseudomonas, shigella, and *E coli*.<sup>27</sup>

So what is the source of serum IgA antibody to klebsiella in patients with ankylosing spondylitis? Our data suggest that the antibody is not of mucosal origin; it is possible that the antibody is formed in the lymph nodes draining the gut, as Ebringer has suggested.<sup>11</sup> The question could be resolved by determining whether circulating IgA antibody to klebsiella contains a secretory component.

Most patients with ankylosing spondylitis in this study had abnormal intestinal permeability, though there was not a significant correlation between the sugar permeability ratio and levels of serum IgA antibody to klebsiella. Increased permeability to probe molecules (such as sugars) may not necessarily be accompanied by increased permeability to other large molecules, such as bacterial antigens.<sup>28</sup>

Smith *et al*<sup>15</sup> reported that abnormal intestinal permeability (using polyethylene glycol (PEG) 400 as the probe molecule) in ankylosing spondylitis is independent of NSAID use. In support of this, we found abnormal intestinal permeability in two patients who had not been receiving NSAIDs at the time of study, but it should be noted that the small intestinal effects of NSAIDs have been reported to persist for up to 18 months after discontinuation of treatment.<sup>28</sup>

Mielants *et al*<sup>17</sup> performed ileocolonoscopies on a large number of patients with ankylosing spondylitis; they reported 'Crohn's-like' histological lesions in most of the patients. These lesions were particularly common in patients with peripheral joint disease, and the degree of intestinal inflammation reflected the clinical activity of the spondylitis. These workers suggest that exogenous factors causing gut inflammation lead to a disturbed permeability of the gut wall or to a deficient local immunological mechanism allowing antigens to enter the circulation, inducing joint and tendon inflammation. Gut lavage fluid IgG levels (a sensitive marker of disease activity in inflammatory bowel disease<sup>20</sup>) in this group of 14 patients with ankylosing spondylitis were similar to control subjects, indicating that clinically important ileitis was not present in this group. One patient, however, did have a markedly increased whole gut lavage fluid IgG level, and it is noteworthy that this particular patient had active disease with an ESR of 57 mm/hour.

Levels of serum IgA antibody to klebsiella in the patients with ankylosing spondylitis significantly correlated with the ESR and the total serum IgA, in agreement with the antibody studies of Ebringer *et al*,<sup>22</sup> who found high antibody levels mainly in those patients with active disease.

This is the first report of the intestinal immune response in ankylosing spondylitis. Our findings do not entirely preclude a pathogenic role for *Klebsiella pneumoniae* in ankylosing spondylitis; though we have shown that serum IgA antibodies to klebsiella are not specific to ankylosing spondylitis, such antibodies may be pathogenic only in HLA-B27 positive subjects. The fact that intestinal levels of antibody to klebsiella were not increased (compared with controls) in the patients with ankylosing spondylitis might reflect the fact that the initial intestinal infection with the organism, and the subsequent immune response, could have occurred long before the time of study. There is, however, some evidence that previous gastrointestinal infections with bacterial microorganisms may leave 'footprints' in the form of persistent secretory antibodies; for example, secretory antibodies following salmonella gastroenteritis persist one year after clinical infection, long after serum antibodies are undetectable.<sup>29</sup> If there is a prolonged mucosal 'immunological memory', then it would be expected that mucosal antibodies to klebsiella should persist.

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