class of road user and other criteria. The Department of Transport could use this system and the method of presenting data used by the Finnish government to provide a much better record of the deaths in road accidents and to identify the classes of road users, especially the innocent victims.[16 17] This information could be used at both local and national levels to assess the effects of alcohol policies and for public information and road safety campaigns.

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Raised titres of anti-klebsiella IgA in ankylosing spondylitis, rheumatoid arthritis, and inflammatory bowel disease

R COOPER, S M FRASER, R D STURROCK, C G GEMMELL

Abstract

Serum titres of IgA are raised in ankylosing spondylitis and increased titres of antibodies to klebsiella have also been reported. The humoral response was investigated in ankylosing spondylitis and other inflammatory disorders. IgA antibodies to klebsiella pneumoniae K43 were measured in patients with ankylosing spondylitis, Crohn's disease, ulcerative colitis, and rheumatoid arthritis and in controls. Significantly raised median titres of anti-klebsiella IgA, measured as optical density at 405 nm with an enzyme linked immunosorbent assay (ELISA), were seen among the patients with ankylosing spondylitis (0·7), Crohn's disease (0·8), rheumatoid arthritis (0·6), and ulcerative colitis (0·8) compared with controls (0·4). Activity of disease in ankylosing spondylitis and titres of anti-klebsiella IgA were not correlated. In contrast, titres of anti-klebsiella IgM were significantly lower in patients with ankylosing spondylitis and ulcerative colitis.

The increase in the titres of anti-klebsiella IgA may be due to increased permeability of the gut to bacterial antigens, leading to an increased IgA response in the gut mucosa and permitting the release of IgA into the circulation. As the increased antibody titres were seen in Crohn's disease and rheumatoid arthritis as well as in ankylosing spondylitis the response may be non-specific, occurring because of possible underlying inflammatory bowel disease in these conditions.

Department of Bacteriology and Centre for Rheumatic Diseases, Glasgow Royal Infirmary, Glasgow G4 0SF

R COOPER, BSC, research assistant
S M FRASER, MB, MRCP, registrar in rheumatology
R D STURROCK, MD, FRCP, senior lecturer and consultant
C G GEMMELL, PHD, MRCPATH, senior lecturer and medical microbiologist

Correspondence to: Dr Sturrock.

Introduction

Recent reports have shown an association between the activity of disease in patients with ankylosing spondylitis and the faecal carriage of *Klebsiella pneumoniae*.[1 2 3] Serum titres of IgA are also raised in ankylosing spondylitis.[4] Humoral immune responses in the gut are mainly of the IgA type; some workers found increased titres of klebsiella antibodies in patients with ankylosing spondylitis and suggested that klebsiella may play a part in the pathogenesis of this disease.[5 6] We set out to investigate the humoral immune response to klebsiella in ankylosing spondylitis and other inflammatory disorders, and we report our preliminary results.

Patients and methods

We obtained serum from 64 patients with ankylosing spondylitis, 25 with rheumatoid arthritis, 30 with Crohn's disease, 20 with ulcerative colitis, and 35 controls. The controls were selected from healthy hospital staff who did not give a history of arthritis or inflammatory bowel disease. They were matched as far as possible by sex and decade of age with the patients. The patients were attending outpatient clinics and seen consecutively.

The patients with ankylosing spondylitis were divided into three groups according to the grade of activity of their disease: active, probably active, and inactive. Active disease was defined as synovitis of peripheral joints, erythrocyte sedimentation rate $\geq$30 mm/h, morning stiffness lasting for 30 minutes or more, and uveitis; probably active as back pain with stiffness and regular treatment with non-steroidal anti-inflammatory drugs; and inactive as occasionally taking analgesics or non-steroidal anti-inflammatory drugs. Concentrations of IgA and C reactive protein were measured as laboratory criteria of disease activity in these patients.[7]

ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA)

Kl pneumoniae K43 (National Collection of Type Cultures 9163) was grown overnight in minimal salts medium supplemented with 11 mmol/l glucose. The bacteria were washed in 0·9% (w/v) saline and inactivated with 1% (v/v) buffered formalin overnight at room temperature. The bacterial

suspension was then washed three times in saline and resuspended to a concentration of 10^{11} colony forming units/l and stored at −20°C until required.

Before use the bacterial suspension was centrifuged and resuspended to its original volume in 0·2 mol/l sodium bicarbonate-carbonate buffer (pH 8·6), and of this suspension 200 µl was absorbed on to flat bottomed microtitre plates (Sterilin) overnight at 37°C. The suspension was decanted, and the wells were washed four times in phosphate buffered saline supplemented with 0·05% (w/v) polysorbate 210, 0·5% (w/v) bovine serum albumin and 0·05% (w/v) sodium azide.

Serum samples were diluted 1 in 10 in phosphate buffered saline (supplemented with 0·5% (w/v) bovine serum albumin and 0·05% (w/v) sodium azide) and 100 µl amounts of each sample were added in triplicate to the wells. Control wells containing serum alone were included for each sample. After two hours' incubation at 37°C the plates were washed. Goat antihuman IgA conjugated to alkaline phosphatase (Sigma) was diluted 1 in 1000 in phosphate buffered saline and 100 µl added to each well; the plates were incubated for two hours at 37°C. After another four washes, 100 µl p-nitrophenol substrate (Sigma) were added and the colour developed for 30 minutes at 37°C. The reaction was stopped with the addition of 100 µl 3 mol/l NaoH. The optical density was measured spectrophotometrically at 405 nm by a Titertek Multiskan system. Results were obtained by subtracting the optical density of the serum in the untreated wells from that in the wells receiving conjugate and substrate.

The specificity of the assay was assessed by absorption studies; separate serum samples were incubated overnight at 40°C on a rotator with *Kl pneumoniae* K43, *Escherichia coli*, and *Pseudomonas aeruginosa*. The bacteria were removed by centrifugation before testing the samples for the presence of antibody to *Kl pneumoniae* K43.

Titres of anti-klebsiella IgG and IgM were measured in the same way with appropriate conjugates.

STATISTICS

The results in the different groups were compared by Wilcoxon's rank sum test and the Mann-Whitney U test and the relation of serum IgA with other biochemical variables by the Spearman Rank Correlation test.

Results

Fig 1 shows the titres of specific anti-klebsiella K43 IgA in serum samples taken from patients in the four groups. Significantly raised median titres were seen in all groups of patients compared with controls separately (ankylosing spondylitis 0·7, $p<0·0001$; rheumatoid arthritis 0·6, $p<0·01$; Crohn's disease 0·8, $p<0·05$; and ulcerative colitis 0·8, $p<0·05$, Mann-Whitney U test) and collectively $p<0·05$. The data on patients with ankylosing spondylitis were analysed according to whether or not the disease was active, probably active, or inactive. Anti-klebsiella IgA titres in all three states were significantly raised when compared with those of the healthy controls but did not differ significantly between each group (table I). There

TABLE I—*Serum titres of anti-klebsiella K43 IgA in 59 patients with ankylosing spondylitis and controls*

	Serum IgA titre (absorbance at 405 nm)			
	Active disease (n=20)	Probably active disease (n=20)	Inactive disease (n=19)	Controls (n=35)
Median	0·8***	0·6*	0·7**	0·4
Interquartile range	0·6-1·0	0·5-0·9	0·5-1·0	0·3-0·7

*p=0·01, **p=0·0005, ***p=0·0003 compared with controls.

TABLE II—*Serum titres of anti-klebsiella K43 IgM in patients with inflammatory disease and controls*

	Serum IgM titre (absorbance at 405 nm)				
	Ankylosing spondylitis (n=64)	Crohn's disease (n=30)	Rheumatoid arthritis (n=25)	Ulcerative colitis (n=20)	Controls (n=35)
Median	0·4**	1·0	0·4	0·4*	0·7
Interquartile range	0·2-0·6	0·5-1·3	0·3-0·8	0·2-0·6	0·4-1·0

*p<0·05, **p<0·001 compared with controls.

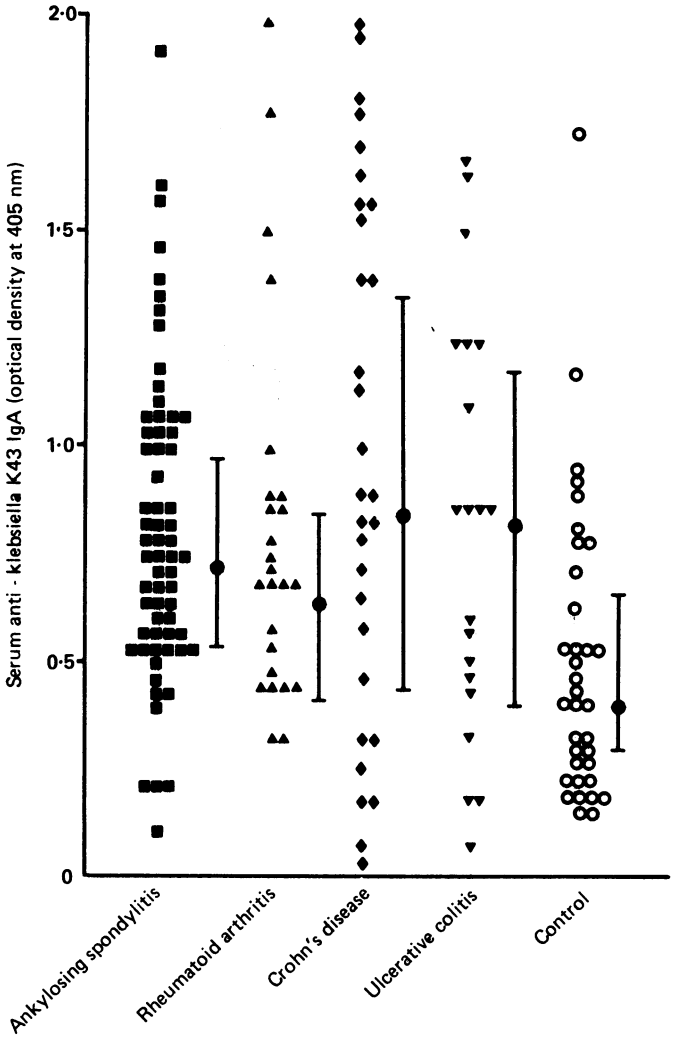

FIG 1—Serum titres of anti-klebsiella K43 IgA in groups of patients and controls. ●=Median; vertical bar=interquartile range.

was no correlation between the titres of anti-klebsiella IgA and the erythrocyte sedimentation rates or C reactive protein concentrations in the patients with ankylosing spondylitis (fig 2; Spearman rank test). The IgA antibodies against *Kl pneumoniae* K43 cells could be absorbed out by cells of the homologous bacterium but not by those of *E coli* or *P aeruginosa*.

There was no significant difference in the titres of anti-klebsiella IgM antibodies in patients with ankylosing spondylitis, Crohn's disease, and rheumatoid arthritis, but they were significantly lower in ankylosing spondylitis and ulcerative colitis when compared with the controls (Table II).

Discussion

The association between ankylosing spondylitis and klebsiella has been intensively investigated, with conflicting results. Ebringer *et al* found an increase in klebsiella in faecal cultures of patients with the active disease,[1] and sequential studies showed that positive results for cultures for klebsiella in patients with inactive disease were associated with subsequent development of the active form.[8,9] Others, however, found no correlation between the faecal carriage of klebsiella and disease activity.[10,11] Our patients with ankylosing spondylitis showed an association of carriage of *Klebsiella sp* with the disease of only 10%[12] compared with a group of community controls, whose incidence of carriage was 9·7%.

Trull *et al* found increased titres of klebsiella IgA antibodies in active ankylosing spondylitis and suggested that these patients were reacting in a similar immunological manner to klebsiella as patients with reactive arthritis after infections with *Yersinia enterocolitica*.[6] As active disease occurred during periods when titres of anti-

klebsiella IgA were raised this suggested that exacerbations of the disease may be triggered by klebsiella antigen present at the mucosal surface of the gut. Another study showed that patients with ankylosing spondylitis had raised serum antibody titres to various other enterobacteria, including salmonella, shigella, and yersinia, suggesting the presence of a common antigenic trigger.[13] We could not confirm the relation between the activity of the disease in ankylosing spondylitis and klebsiella antibody titres, either by clinical or laboratory criteria of disease activity.

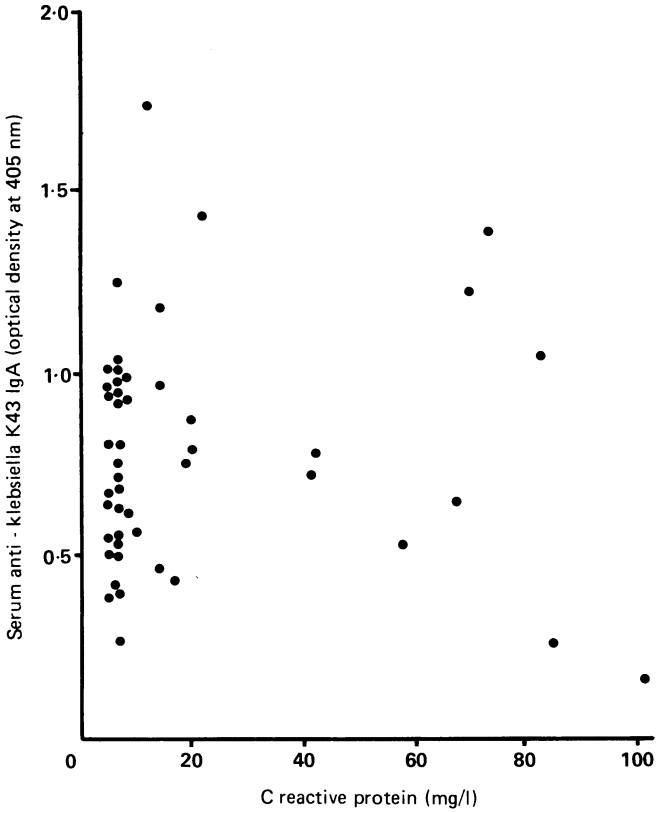

FIG 2—Titres of anti-klebsiella K43 IgA in ankylosing spondylitis with corresponding concentrations of C reactive protein.

We investigated the immune response to *Kl pneumoniae* in the serum of patients with various inflammatory disorders. The results showed that klebsiella IgA antibodies were significantly raised in ankylosing spondylitis, Crohn's disease, ulcerative colitis, and rheumatoid arthritis when compared with controls but that titres of klebsiella IgG and IgM antibodies were not increased. Titres of anti-klebsiella 43 IgM were lower in the patients with ankylosing spondylitis and ulcerative colitis, but the reasons for this are not clear. The increase in the IgA titres may be due to increased permeability of the gut to bacterial antigens, leading to an increased mucosal IgA response in the gut, thus allowing the release of IgA into the circulation. As increased antibody titres were seen in Crohn's disease, ulcerative colitis, and rheumatoid arthritis as well as in ankylosing spondylitis a non-specific immunological response may occur when a disorder of the gut exists.

In Crohn's disease the small and large bowel may be inflamed, and the use of non-steroidal anti-inflammatory drugs in rheumatoid arthritis can cause increased permeability of the small bowel.[14-16] The increase in serum IgA in patients with ankylosing spondylitis could well be due to the effects of these drugs or to low grade inflammatory bowel disease and is found in all patients with ankylosing spondylitis, irrespective of the activity of disease. If there is a non-specific antibody response as a result of increased gut permeability an increased response to other enteric bacteria would be expected. Ebringer *et al*, however reported a selective antibody response to proteus in patients with rheumatoid arthritis, which was not observed in those with ankylosing spondylitis,[17] but these observations await confirmation. Clearly, the relation between the expression of disease, the permeability of the bowel, and the immune response to the flora of the gut in chronic inflammatory joint disease merits further clarification.

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ONE HUNDRED YEARS AGO

CREMATION in Italy, says a correspondent of the *Times*, has not for the last two years or so made so much headway as at first. The example of Milan in 1876 soon found many imitators, especially in the northern and central provinces of Italy, and there are now something like thirty-two societies and committees for favouring cremation, though they have not all got crematories of their own yet. The number of persons burnt last year was only 165, as against 181 in 1886. The Pisa Society, however, has not yet sent in its statistics for last year, and crematoriums will be opened in the course of the present year at Turin, San Remo, Verona, Bologna, Pavia, and one or two other towns. Of the total number of 952 cremations that have occurred in 17 cities in Italy, since 1876, as many as 518 have taken place in Milan, and 155 in Rome. The new crematorium at Milan is situated at the extreme end of the Campo Santo, just outside the walls of the city. The temple, as it is called, is a building in the Doric style, constructed of stone, and having an open façade supported by columns, from behind which rises a tower which, as seen from the outside, looks as if it formed part of the temple, although in reality it stands quite by itself, and is the chimney. The inside of the building is divided into several rooms, in the first of which the religious rites are performed; its walls are lined with funeral urns containing the ashes of many of those who have been cremated at Milan. There is a separate room in which the bodies are placed pending cremation, and a third in which the relatives and friends spend the two hours occupied by the cremation itself. There are two furnaces—one being for general use, and the other for the bodies of persons who have died of contagious diseases and are not natives of Milan. The body is not visible to the onlookers when being put into the furnaces, nor are the ashes afterwards.

(*British Medical Journal* 1888;i:707)