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Secretory IgA: immune defence pattern in ankylosing spondylitis and klebsiella

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SUMMARY Saliva secretory IgA (sIgA), secretory component (SC); serum immunoglobulins (IgG, IgA, IgM), complement (C_3, C_4), C-reactive protein (CRP), and erythrocyte sedimentation rate (ESR) were performed in 32 patients with ankylosing spondylitis and 29 normal controls. They were investigated for carriage in the faeces of *Klebsiella* spp. on 3 occasions over the previous months. Throat swabs and urine were cultured at the same time as immunological estimations were done. 24-hour urine sIgA specimens were studied in 13 patients and 12 normal controls. Significantly raised mean values of saliva sIgA and serum IgG, IgA, C_3, and C_4 were found in patients with raised values of serum ESR and CRP levels when correlated with controls. Raised values of sIgA in saliva, which is an important factor of the local immune defence mechanism of mucosal surfaces, suggests the presence of an antigenic stimulus from the gastrointestinal system in ankylosing spondylitis during activity of disease.

An infective aetiology for ankylosing spondylitis (AS), especially an association with genitourinary infection has been postulated for many years.[1-4] More recently an increased isolation of klebsiella from the faeces of AS patients during active phases of disease has been reported,[5 6] though others have not been able to confirm these results.[7] The Leeds group has demonstrated the association in these patients to be particularly marked in those with uveitis.[8]

The association between AS and HLA B27[9 10] has led many workers to suggest that the pathogenesis of AS might involve immune response genes, although as yet no disease susceptibility locus or immune response gene has been identified.

Raised levels of serum IgA and C_4 in patients with AS have been reported.[11] Individuals with HLA B27 positive AS have higher mean serum concentrations of IgG and IgA than normal controls[12] and than their B27 positive and negative relatives.[13]

In human serum IgA is 85% monomeric, 10-15% polymeric, and 1% sIgA. sIgA levels tend to rise in patients with a variety of mucosal inflammatory diseases.[14] In man monomer is synthesised mainly in nonmucosal lymphoid tissues, while both sIgA and polymeric IgA come from mucosal sites, especially the gut,[15] which has an integral and important role in the immune defence of the gastrointestinal tract.

sIgA is the predominant immunoglobulin in secretory fluids of the body. It has been shown that this immunoglobulin is found in milk, saliva, tears, urine, cervicovaginal fluids, amniotic fluids, and gastrointestinal secretions.[16 17]

The IgA molecule in external secretions has several features that distinguish it from its serum counterpart. Serum IgA in man is predominantly a 7S monomer (molecular weight 160 000), while the sIgA is an 11S dimer (molecular weight 400 000) consisting of 2 units of 7S IgA together with 2 non-immunoglobulin components, 'secretory component' (molecular weight 70 000) and a 'J' chain (molecular weight 15 000).[18]

This report is concerned with an investigation into the nature of the local immune defence system in ankylosing spondylitis.

Patients and methods

Thirty-two outpatients with ankylosing spondylitis and 29 normal controls have entered the study.

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Table 1 shows the age and sex distribution of the patients and controls. The diagnosis of AS was made by the New York criteria.[19] Three patients did not have the HLA B27 antigen and the test was not performed in 4 cases. Patients with inflammatory bowel disease and Reiter's disease were excluded. Controls were selected from medical staff and other volunteers.

All patients and controls had been asked to post a stool specimen passed within the previous 24 hours on 3 occasions over a 2-month period with 4-week intervals. Each patient filled in a diary over the time of the study to assess anti-inflammatory drug requirement, duration of early morning stiffness, severity of back pain, peripheral joint symptoms, eye symptoms, and any infectious disease or antibiotic therapy. All controls were provided with a special questionnaire to indicate any infectious disease or antibiotic therapy over the same period.

At the end of 2 months patients and controls were called to a special clinic for collection of saliva, blood, midstream specimen of urine, and throat swab between 1 pm and 4 pm. Assessment of clinical disease activity of patients was based on the criteria which have been described before.[8]

SALIVA SAMPLES

Parotid saliva was collected by using modified Curby cups[20] from the right side in the majority of cases. (These cups were specially made for the study by the Department of Oral Biology, Dental School, Leeds.) Saliva was stimulated by approximately 10 ml 10% citric acid at the beginning of the collection. 10 ml of saliva was collected from all except 3 patients, including the first 0·5 ml unstimulated specimen which was present in the parotid duct prior to the stimulation. No sodium azide preservatives were added, but the samples were immediately stored at −20°C.

All samples were concentrated 25 and 50 times in Minicon B15 concentrators (Amicon Corporation). The levels of sIgA in the saliva were estimated by a modified single radial immunodiffusion technique[21] using Dako commercially available antiserum (Dako rabbit IgS to colostrum IgA—α chain and secretory piece). Hoechst radial immune diffusion plates were used as templates. 5·2 ml of 1% agarose in barbitone acetate buffer and 110 ml antiserum were used to pour on the plates. Wells were punched with a 2 mm diameter well punch and filled with a 5 μl volume of specimen. Hoechst standard human sera were used as standards. A correction was made by a factor (1·72) for the slower mobility of 11S salivary IgA as compared to the 7S serum IgA of the standards.[22] SC levels in the saliva were measured by single radial immunodiffusion technique.[23] 400 μl concentration of Nordic commercially available antisera (antisecretory piece—free and bound—produced in sheep) were used. Plates were prepared in the same way as described for sIgA. No standards were used for SC estimation.

Table 1 *Age and sex of patients and controls*

	n	*Male*	*Female*	*Mean age range in years*
Patients	32	28	4	42·41 (25–61)
Controls	29	26	3	36·07 (23–65)

URINE SAMPLES

24-hour urine samples were collected from 13 patients and 12 controls for sIgA estimation. Samples were dialysed in Visking 36/32 membranes in the cold and were concentrated 1000 times by the use of a freeze-dryer.[24] All samples were stored at −70°C until used. No 24-hour specimen of urine contained more than 80 mg of protein by folin or biuret reactions.

The levels of sIgA in the urine were carried out by the same method as was used for saliva sIgA.

SERUM SAMPLES

Serum IgG, IgM, and C_3 and C_4 estimations were determined by the radial immunodiffusion technique with commercially available immunodiffusion plates and protein standards (Behringwerke-Marburg). Serum ESR was measured by the Westergren method. The normal hospital service was used. Serum CRP estimations were determined by the radial immunodiffusion technique at the Clinical Pharmacology Unit, Royal Bath Hospital.

BACTERIOLOGY

All faecal samples, urines, and throat swabs were cultured on MacConkey inositol carbenicillin agar (MIC) and citrate agar plates and in citric broth. The broths were subcultured to MIC and citrate agar plates after incubation for 48 hours. *Klebsiella* spp. were identified as previously.[25] Throat swabs were also cultured on blood agar and then examined for the presence of beta-haemolytic streptococci.

Results

SERUM

The results of serum mean IgG, IgA, IgM, C_3, and C_4 estimations are shown in Table 2. The mean serum IgA, C_3, and C_4 levels in AS patients were significantly higher than in normal controls. There was a slight rise in mean serum IgG and IgM estimations in AS patients when compared with normal controls, but this difference was not statistically significant.

Table 2 *Mean (± SD) serum immunoglobulins and C_3 and C_4 levels in normal controls and AS patients*

	Controls		*As patients*		
Serum protein	*Mean±SD g/l*	*Number of estimations*	*Mean±SD g/l*	*Number of estimations*	*Statistical significance*
IgG	11·07±3·41	28	13·83±7·48	32	NS
IgA	1·83±0·64	28	2·62±1·34	32	P<0·01 $t = 2·83$
IgM	1·13±0·38	28	1·35±0·62	32	NS
C_3	0·84±0·22	28	1·04±0·35	32	0·02>p>0·01 $t = 2·62$
C_4	0·25±0·09	28	0·34±0·14	32	P<0·01 $t = 2·85$

Table 3 *Serum mean (±SD) IgG, IgA, C_3, and C_4 levels in normal controls compared with AS patients with raised ESR and CRP levels*

	Controls		*As patients*		
Serum protein	*Mean±SD g/l*	*Number of estimations*	*Mean±SD g/l*	*Number of estimations*	*Statistical significance*
IgG	11·07±3·41	28	15·99±9·62	16	0·02>p>0·01 $t = 2·46$
IgA	1·83±0·64	28	3·25±1·41	16	P<0·001 $t = 4·56$
C_3	0·84±0·22	28	1·04±0·33	16	0·02>P>0·01 $t = 2·50$
C_4	0·25±0·09	28	0·37±0·14	16	P<0·01 $t = 3·35$

The increases in serum IgG, IgA, C_3, and C_4 estimations in AS patients were seen even more strikingly in those with raised serum ESR and CRP levels (Table 3).

SALIVA

The results of mean saliva sIgA and SC estimations are shown in Table 4. There was a slight rise in mean saliva sIgA and SC estimations in AS patients when compared with normal controls, but this difference was not statistically significant. The patients were divided into 2 groups according to their serum ESR and CRP levels. Saliva sIgA and SC in AS patients with raised ESR and CRP levels was significantly higher than the other group, but the difference was statistically significant only for sIgA (Table 5).

URINE

The mean urine sIgA in AS patients was 2·26

Table 4 *Mean (± SD) saliva sIgA and SC levels in normal controls and AS patients*

	Controls		*AS patients*		
Parotid secretion	*Mean±SD*	*Number of estimations*	*Mean±SD*	*Number of estimations*	*Statistical significance*
sIgA (mg/dl)	4·33±1·71	29	4·59±1·87	29	NS
SC (d^2)	60·73±17·58	29	65·89±18·01	29	NS

Table 5 *Mean (± SD) saliva sIgA and SC distribution in AS patients correlated with serum ESR and CRP levels. Group A: ESR<15 mm/h, CRP<0·50 mg/100 ml (5 mg/l). Group B: ESR>15 mm/h, CRP>0·50 mg/100 ml (5 mg/l).*

	Group A		*Group B*		
Parotid secretion	*Mean±SD*	*Number of estimations*	*Mean±SD*	*Number of estimations*	*Statistical significance*
sIgA (mg/dl)	3·76±1·22	12	5·48±2·00	15	P<0·02 $t = 2·60$
SC (d^2)	58·65±11·58	12	71·70±19·19	15	P = 0·05 $t = 2·06$

SI conversion: mg/l = mg/dl × 10. d^2 = Diameter squared.

Table 6 Klebsiella *spp. (Kl) carriage in normal controls and AS patients correlated with clinical disease activity over a 2-month period*

	Kl (+)	*n*	*Per cent.*
Normal controls	15	29	51
Active (AS patients)	3	6	50
Probably active (AS patients)	12	21	57
Inactive (AS patients)	1	5	20
Total (AS patients)	16	32	50

(± 2·05) mg/24 h compared with 2·53 (± 1·24) mg/24 h in controls, and the difference was not statistically significant.

BACTERIOLOGY

Thirty-two patients provided 87 faecal specimens, of which 26 (30%) gave positive cultures for *Klebsiella* spp. compared with 23 out of 79 (29%) of the specimens from the 29 controls. 16 patients (50%) and 15 controls (51%) had *Klebsiella* spp. isolated from the faeces on at least one occasion over the 2-month period of the survey. Clinical disease activity and faecal klebsiella carriage are compared in Table 6.

AS patients with raised serum ESR and CRP were divided into 2 groups according to their klebsiella carriage and were compared for their serum immunoglobulins and C_3, C_4 concentrations. Serum mean IgG and IgA, especially IgG, was higher and C_4 was lower in the klebsiella-negative group than in klebsiella-positive individuals, but the difference was not statistically significant (Table 7).

No mid urine specimens showed any significant bacterial growth.

β-haemolytic streptococcus was isolated from the throat of 1 of the patients and 2 of the controls. There was no significant change in immunological estimations of these cases.

Discussion

Raised Serum IgG, IgA, and C_4 levels in AS patients, especially in those with raised serum ESR and CRP levels is in keeping with the findings of previous studies.[11–13] There was also a significant rise in serum C_3 in our study. A good correlation between raised values of serum ESR, CRP, and disease activity has been shown in AS patients.[26] Mean serum IgA in patients with raised serum ESR and CRP was greater than in those with lower values. Mean serum IgG and C_4 levels were raised in patients with a raised ESR and CRP, but these differences were not statistically significant.

None of the patients or the controls had sIgA deficiency. Mean saliva sIgA was slightly higher in AS patients than in controls. More strikingly, however, patients with raised serum ESR and CRP levels had statistically significant higher levels of saliva sIgA than those with low levels of acute-phase proteins. Moreover, the same group of AS patients with high sIgA in their saliva had significantly high mean serum IgA correlated with disease activity. None of the AS patients showed any difference in their mean urine sIgA compared with controls.

We have found a similar overall frequency of *Klebsiella* spp. carriage in AS patients compared with controls. Positive cultures for *Klebsiella* spp. were more frequent in AS patients who had clinically active disease (50%) than in patients with inactive disease (20%), but the latter represents only 1 of 5 patterns. A comparison of klebsiella carriers and klebsiella-negative patients in an active phase showed no significant difference between the immunological findings of the 2 groups.

It may be concluded that the raised serum IgA in AS patients comes from gut mucosa, which strongly suggests, klebsiella or not, an antigenic triggering agent from the gastrointestinal system in AS patients. Raised serum IgG levels suggest further systemic antibody response as well as a local immune response in these patients.

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Table 7 *Serum mean (± SD) IgG, IgA, and C_4 levels in AS patients with raised serum ESR, and CRP levels and* Klebsiella *spp. distribution*

Kl(+) *AS patients*			*Kl*(−) *AS patients*		
Serum protein	*Mean ± SD g/l*	*Number of estimations*	*Mean ± SD g/l*	*Number of estimations*	*Statistical significance*
IgG	13·91 ± 9·25	8	18·06 ± 10·15	8	NS
IgA	3·16 ± 1·70	8	3·34 ± 1·16	8	NS
C_4	0·45 ± 0·14	8	0·29 ± 0·10	8	NS

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