Association of inflammation with raised serum IgA in ankylosing spondylitis

P. COWLING,¹ R. EBRINGER,² AND A. EBRINGER¹

From the ¹Immunology Unit, Department of Biochemistry, Queen Elizabeth College, London W8, and the ²Department of Rheumatology, Middlesex Hospital, Arthur Stanley House, Tottenham Street, London W1P 9PG

SUMMARY Serum immunoglobulins were measured in 122 patients with ankylosing spondylitis (AS) during various phases of disease activity and compared to those in 58 healthy subjects. The mean serum IgA was 38% higher in patients (306.9 mg/dl) than in controls (222.7 mg/dl) ($P < 0.005$), but there was no significant difference in IgG and IgM levels. Increased IgA was associated with laboratory parameters of active inflammatory disease. The mean IgA in patients having an erythrocyte sedimentation rate (ESR) equal to or greater than 15 mm/h was 369 mg/dl, 65% higher than in controls ($P < 0.001$), whereas there was no significant difference between controls and patients with an ESR of less than 15 mm/h. The mean IgA in patients having a C-reactive protein (CRP) level equal to or greater than 15 µg/ml (15 mg/l) was 387.8 mg/dl, 74% higher than in controls ($P < 0.001$), and again there was no significant difference between controls and patients with CRP levels less than 15 µg/ml. (SI conversion: g/l = mg/dl $\times$ 0.01). It is suggested that selective increase of serum IgA occurs predominantly during phases of active inflammatory disease in AS, and this finding is compatible with the concept of a microbial triggering agent acting across an IgA secreting organ such as the gut.

There is evidence that gastrointestinal infection may be an important aetiological factor in ankylosing spondylitis (AS). Sacroiliitis and AS are common sequelae in persons who develop arthritis or Reiter's disease after dysenteric infection with salmonella, shigella, or *Yersinia enterocolitica*.¹ We have reported an increased isolation of *Klebsiella pneumoniae* from the faeces of AS patients before and during active phases of the disease² or during episodes of acute anterior uveitis.³ Investigation by lymphangiography has shown that pelvic and paraspinal lymphadenopathy precedes the radiological changes in AS.⁴ Raised levels of serum IgA in patients with AS have been reported⁵⁻⁸. Plasma cells in the gut associated lymphoid tissue are the major source of serum IgA,⁹ and investigations in animals raised in germ-free conditions have shown that after oral immunisation the main specific plasma cell response is IgA in character and nearly all the circulating specific antibody is IgA.¹⁰

We report here our studies on serum immunoglobulin levels in AS.

Patients and methods

Blood was obtained from 122 AS patients during varying phases of disease activity. A total of 221 patient sera were randomly selected from a bank of stored sera, each patient being selected on average twice (range 1-5). Each patient visit was considered as 1 separate event, the shortest interval between visits being 1 month. The New York criteria were used for the diagnosis of AS.¹¹ Patients with inflammatory bowel disease were excluded from the study. The mean age of all patients was 34.5 years (range 11-68), and 29 were women. Of 117 patients tested 110 were HLA B27 positive (94%). Single serum samples were obtained from 58 healthy subjects corresponding in age and sex distribution to the AS patients and who had no history of arthritis or backache.

Serum IgA, IgG, and IgM estimations were determined by the radial immunodiffusion technique with commercially prepared immunodiffusion plates and protein standards (Behringwerke, Marburg/Lahn). Blood for the erythrocyte sedimentation rate (ESR) was taken at each patient visit and

measured by the Westergren method. Serum C-reactive protein (CRP) estimations were determined by the radial immunodiffusion technique with commercial antisera (Hoechst), and each estimation was the mean of at least 2 separate measurements. All measurements were carried out in code without knowledge of the patient's clinical status.

Results

IgA

The results of the serum immunoglobulin estimations are shown in Table 1. The mean serum IgA in AS patients was 307 mg/dl compared to a mean serum IgA of 223 mg/dl in the control group. This is an increase of 38% and is statistically significant ($t=3.14$, $P<0.005$). (SI conversion: $\text{g/l}=\text{mg/dl} \times 0.01$). There was a slight rise in mean serum IgG and IgM estimations in AS patients when compared to control subjects, but this difference was not statistically significant (Table 1).

In an endeavour to study further the distribution pattern of serum IgA, AS patients were divided into 2 groups according to their ESR: one group consisting of patients with an ESR below 15 mm/h ('normal ESR') and the other group consisting of patients having an ESR of 15 mm/h or more ('elevated ESR'). The mean serum IgA in patients having an 'elevated ESR' was 369 mg/dl (Fig. 1A), while in AS patients having a 'normal ESR' it was 236 mg/dl, and this difference is statistically significant ($t=5.64$, $P<0.001$). The mean serum IgA in patients with an 'elevated ESR' was 65%, higher than the mean serum IgA in control subjects ($t=4.52$, $P<0.001$). Furthermore there was no significant difference between the mean serum IgA in AS patients with a 'normal ESR' and the mean serum IgA in control subjects (Fig. 1A).

Samples for CRP estimations were selected randomly from the pool of patient sera available. The patients were divided into 2 groups according to their CRP level: one group consisting of patients with a CRP below 15 $\mu\text{g/ml}$ and the second group having a CRP of 15 $\mu\text{g/ml}$ (15 mg/l) or more (Fig.

1A). It was considered that patients having the higher level of CRP were more likely, as a group, to be in an active inflammatory phase of the disease. The mean serum IgA in patients having an elevated CRP was 387.8 mg/dl, while in patients having the lower CRP (Fig. 1A) it was 236.5 mg/dl, and this difference is statistically significant ($t=3.68$, $P<0.001$). The mean serum IgA in patients with an

Fig. 1 Serum IgA (mean $\pm$ standard error) (Fig. 1A), IgG (Fig. 1B), and IgM (Fig. 1C) measured in healthy control subjects and AS patients. Mean serum immunoglobulin levels in patients with elevated erythrocyte sedimentation rate ($\text{ESR} \geq 15$ mm/h) and elevated C-reactive protein levels ($\text{CRP} \geq 15$ $\mu\text{g/ml}$) were also compared to sera from patients having normal ($\text{ESR} < 15$ mm/h) or low ($\text{CRP} < 15$ $\mu\text{g/ml}$) values.

Table 1 The mean ($\pm$ SEM) serum immunoglobulin levels in healthy control subjects and AS patients

	Controls		Ankylosing spondylitis patients		Statistical significance
	Mean $\pm$ SE (mg/dl)	Number of estimations	Mean $\pm$ SE (mg/dl)	Number of estimations	
Serum IgA	223 $\pm$ 11	58	307 $\pm$ 13	221	$t=3.140$ $P<0.005$
Serum IgG	1263 $\pm$ 36	58	1361 $\pm$ 30	195	NS
Serum IgM	140 $\pm$ 6	58	149 $\pm$ 6	185	NS

SI conversion: $\text{g/l}=\text{mg/dl} \times 0.01$. NS=not significant.

Association of inflammation with raised serum IgA in ankylosing spondylitis 547

elevated CRP value was 74% higher than in control subjects ($t=4.54$, $P<0.001$). Again as in the previous ESR comparison there was no significant difference between the mean serum IgA of the low CRP group and the control subjects.

IgG

The mean serum IgG in AS patients was 1361 mg/dl, which is a rise of 8% compared with the mean serum IgG obtained for control subjects, but this difference is not statistically significant. However, when the AS patients were divided into groups with increased inflammatory activity ($ESR \geq 15$ mm/h and $CRP \geq 15$ μ g/ml), again there was a rise in the mean serum IgG when compared with the level in control subjects (Fig. 1B). The mean serum IgG in patients with an elevated ESR was 1508 mg/dl and this 20% rise, when compared to control subjects, is statistically significant ($t=3.85$, $P<0.001$). The mean serum IgG in patients with an elevated CRP was 1468 mg/dl, and this 16% rise, when compared with the level in control subjects, is also statistically significant ($t=3.40$, $P<0.001$). There was no significant difference between mean serum IgG levels in patients with 'normal' ESR and low CRP values when compared with control subjects.

IgM

The mean serum IgM in AS patients was 149 mg/dl, which is a 6% rise compared to the mean serum IgM obtained for control subjects, but this difference is not statistically significant (Fig. 1C). Furthermore, when patients were divided into groups with increased inflammatory activity, again there was no statistically significant difference between serum IgM levels in patients with elevated ESR or CRP and control subjects (Fig. 1C) or patients with 'normal' ESR and low CRP values.

Discussion

We have confirmed the findings of several previous investigators and shown that serum IgA is raised in AS.⁵⁻⁸ Our further investigations have shown that these elevated IgA levels are associated predominantly with active phases of AS as measured by ESR and CRP. There is also a comparatively smaller but definite rise in serum IgG in active phases of AS. We have previously found that both ESR and CRP correlate with clinical assessment of disease activity.¹² More striking, however, is the observation that there was no elevation in the mean IgA of patients with inactive disease as measured by laboratory parameters.

The origin or site of the inflammatory activity in AS remain obscure. Inflammation obviously must

occur along the axial skeleton and in peripheral joint and eye tissues, but this is unlikely to explain the marked elevation in serum IgA, a class of immunoglobulin produced mainly within the mucosal associated lymphoid tissues of the gastrointestinal tract.⁹

This finding strongly suggests that some external triggering factor is acting across a mucosal surface such as the lower gastrointestinal tract, initiating the rise in IgA and possibly the associated inflammatory disease. We have previously demonstrated an increased isolation of *Klebsiella pneumoniae* from the faeces of AS patients with active joint and eye disease^{2,3} and have also found that elevated ESR and CRP both independently correlated with positive faecal cultures for this micro-organism.¹²

Lymphangiographic studies indicate that regional lymph nodes draining the pelvis, pelvic colon, and rectum, the presacral and para-aortic lymph nodes, become enlarged during the active or progressive phase of AS and before the development of radiographic changes.⁵

Inflammatory changes in the large bowel have been demonstrated in AS by rectal biopsy,¹³ and an association between inflammatory bowel disease and AS has been well established.¹⁴ Even if the large bowel does have an aetiological role in the pathogenesis of AS, it is still unclear why distal sites such as the spine, peripheral joints, and the eye are affected. The lesions may be caused by release of arthritogenic bacterial debris from the gut such as peptidoglycans,¹⁵ but that hypothesis alone cannot explain the strong predisposition by individuals with the tissue antigen HLA B27 for developing AS. One possible explanation is provided by the cross-reactivity or molecular mimicry theory,¹⁶ which suggests that some micro-organisms may carry surface antigens having stereochemical similarity to self-antigen of the host such as HLA B27.¹⁷⁻²⁰

Persistent infection may lead to the production of cross-reacting antimicrobial antibodies which can also act as self-damaging autoantibodies. If these antimicrobial antibodies bind to self-antigen, then tissue damage may follow complement activation, migration of leucocytes to inflamed regions, and release of lysosomal enzymes from inflammatory cells. Such tissue damage may lead to increased production of IgG, as has been shown in other conditions,²¹ and this could explain the slight rise in serum IgG in our AS patients. A similar mechanism may operate in the reactive arthritis of acute rheumatic fever or in the B27 positive arthritis following infection with salmonella, shigella, or *Yersinia enterocolitica*. In these examples raised antibody levels have been found against the primary infecting micro-organisms. Whether such anti-

microbial antibodies can be demonstrated in the raised IgA of patients with active AS remains to be seen. An increase in serum IgA immunoglobulin during active disease would appear to be consistent with the hypothesis that microbial infection in the gut could act as an initiating agent or trigger in the development of AS.

This study was supported by the Arthritis and Rheumatism Council. P. C. was in receipt of an SRC studentship, and we thank Mrs C. Padamsey for typing the manuscript. We are grateful to Dr A. C. Boyle for his help and support.

References

- Aho K, Ahvonen P, Lassus A, Sievers K, Tiilikainen A. Yersinia arthritis and related diseases: clinical and immunogenetic implications. In: Dumonde D C, ed. *Infection and Immunology in the Rheumatic Diseases*. Oxford: Blackwell, 1976: 341-4.
- Ebringer R W, Cawdell D R, Cowling P, Ebringer A. Sequential studies in ankylosing spondylitis: Association of *Klebsiella pneumoniae* with active disease. *Ann Rheum Dis* 1978; 37: 146-51.
- Ebringer R, Cawdell D, Ebringer A. *Klebsiella pneumoniae* and acute anterior uveitis in ankylosing spondylitis. *Br Med J* 1979; i: 383.
- Fournier A M, Denizet D, Delagrèze A. La lymphographie dans la spondylarthrite ankylosante. *J Radiol Electrol Med Nucl* 1969; 50: 773-84.
- Langness U, Banavsky D, Alexopoulos J, Kriegel W, Burger R. Differentiation of inflammation and ossification in spondylarthritis ankylopoietica. *Acta Rheum Scand* 1971; 17: 15-22.
- Veys E M, Van Laere M. Serum IgG, IgM, and IgA levels in ankylosing spondylitis. *Ann Rheum Dis* 1973; 32: 493-6.
- Kinsella T D, Espinoza L, Vasey F B. Serum complement and immunoglobulin levels in sporadic and familial ankylosing spondylitis. *J Rheumatol* 1975; 2: 308-13.
- Nikbin B, Brewerton D A, Byrom N, et al. Lymphocyte function in ankylosing spondylitis. *Ann Rheum Dis* 1975; 34: 49-54.
- Lamm M E. Cellular aspects of immunoglobulin A. *Adv Immunol* 1976; 22: 223-90.
- Crabbé P A, Nash D R, Bazin M, Eyssen H, Heremans J F. Antibodies of the IgA type in intestinal plasma cells of germ free mice after oral or parenteral immunization with ferritin. *J Exp Med* 1969; 130: 723-44.
- Bennet P H, Wood P H N. In: Bennet P H, Wood P H N, eds. *Population Studies of the Rheumatic Diseases*. Amsterdam: Excerpta Medica Foundation, 1968; 456.
- Cowling P, Ebringer R, Cawdell D, Ishii M, Ebringer A. C-reactive protein, ESR, and *klebsiella* in ankylosing spondylitis. *Ann Rheum Dis* 1980; 39: 45-9.
- Jayson M I V, Salmon P R, Harrison W J. Inflammatory bowel disease in ankylosing spondylitis. *Gut* 1970; 11: 506-11.
- Wright V. Seronegative polyarthritis: a unified concept. *Arthritis Rheum* 1978; 21: 619-33.
- Hadler N M, Granovetter D A. Phlogistic properties of bacterial debris. *Semin Arthritis Rheum* 1978; 8: 1-16.
- Ebringer A. The link between genes and disease. *New Scientist* 1978; 79: 865-7.
- Ebringer A, Cowling P, Ngwa Suh N, James D C O, Ebringer R W. Crossreactivity between *Klebsiella aero-*

genes species and B27 lymphocyte antigens as an aetiological factor in ankylosing spondylitis. Abstract in: Dausset J, Svejgaard A, eds. *HLA and Disease*. Paris: INSERM, 1976: 27.

- ¹⁸ Seager K, Bashir H V, Geczy A F, Edmonds J, De Vere-Tyndall A. Evidence for a specific B27 associated cell surface marker on lymphocytes of patients with ankylosing spondylitis. *Nature* 1979; **277**: 68–70.
- ¹⁹ Welsh, J Avakian H, Cowling P, *et al.* Ankylosing spondylitis, HLA-B27 and Klebsiella. I. Crossreactivity studies with rabbit antisera. *Br J Exp Pathol* 1980; **61**: 85–91.
- ²⁰ Avakian H, Welsh J, Ebringer A, Entwistle C C. Ankylosing spondylitis, HLA B27 and Klebsiella. II. Cross-reactivity studies with human tissue typing sera. *Br J Exp Pathol* 1980; **61**: 92–6.
- ²¹ Ebringer A, Doyle A E. Raised serum IgG levels in hypertension. *Br Med J* 1970; **ii**: 146–8.