IgG and IgA immune response against klebsiella in HLA-B27-associated anterior uveitis

A KIJLSTRA,¹ L LUYENDIJK,¹ R VAN DER GAAG,¹ E VAN KREGTEN,²
A LINSSSEN,¹ AND J M N WILLERS²

¹From the Department of Ophthalmology, Netherlands Ophthalmic Research Institute, Amsterdam, and ²the Laboratory of Microbiology, State University of Utrecht, Utrecht, The Netherlands

SUMMARY Enteric infections with Gram-negative bacteria are thought to play an important part in HLA-B27-associated disease such as Reiter's syndrome and reactive arthritis. But the role of bacterial infections in HLA-B27-positive ankylosing spondylitis (AS) and acute anterior uveitis (AU) is still controversial. A special interest has recently been devoted to the role of klebsiella infection in HLA-B27-associated disease. We studied the humoral immune response against a 'cross-reactive' strain of *Klebsiella pneumoniae* in 62 patients with anterior uveitis and 33 healthy controls. The anterior uveitis patients were subdivided into 25 HLA-B27-negative patients without AS (B27⁻ AU⁺ AS⁻), 17 HLA-B27-positive patients without ankylosing spondylitis (B27⁺ AU⁺ AS⁻), and 19 HLA-B27-positive patients with ankylosing spondylitis (B27⁺ AU⁺ AS⁺). Total serum IgA was higher in patients than in controls in both the B27⁺ AU⁺ AS⁺ and B27⁺ AU⁺ AS⁻ patients but not in the B27⁻ AU⁺ AS⁻ group. No abnormalities were observed in the total serum IgG levels. The level of both the IgG and IgA klebsiella antibodies did not differ in the various patient groups tested as compared with the controls. Comparisons between the patient groups showed that the IgG anti-klebsiella response was higher in B27-positive patients without AS than in those with AS. These results suggest that stimulation of mucosal surfaces may play a role in HLA-B27-associated anterior uveitis. Whether klebsiella organisms are involved in this stimulation remains unclear.

Enteric infections with certain Gram-negative bacteria are thought to play an important role in HLA-B27-associated disease.^{1,2} Strong though circumstantial evidence to support the role of Gram-negative bacteria in the initiation of HLA-B27-associated disease is found in Reiter's syndrome and reactive arthritis.³ The role of enteric infections in the pathogenesis of ankylosing spondylitis and HLA-B27-positive acute anterior uveitis is still not clear. Some groups have found an increase in klebsiella in the faeces of patients with anterior uveitis,^{1,4} whereas others were unable to confirm these findings.^{5,6} Similar controversial findings have been obtained in ankylosing spondylitis patients.⁷⁻⁹

Several theories have been proposed to explain the role of Gram-negative bacteria in the pathogenesis of HLA-B27-associated disease. Cross-reactions

between klebsiella and HLA-B27 antigens^{9a} or ligand receptor interactions between bacterial antigens and HLA-B27¹⁰ have been found by some workers but were not reproduced in other laboratories.^{11,12}

Increased serum IgA levels in ankylosing spondylitis^{13,14} suggest that mucosal infections may play a part in the course of the disease. Other studies have shown that klebsiella-specific IgA antibodies were increased in ankylosing spondylitis, and the antibody levels were shown to be correlated with disease activity.¹⁵ Whether the IgA response against klebsiella organisms is also altered in HLA-B27-associated acute anterior uveitis has not been thoroughly investigated until now and was therefore the purpose of the study described here. The results show that, although total serum IgA levels are increased in HLA-B27-positive anterior uveitis, the relative amount of IgA anti-klebsiella antibodies is decreased in comparison with controls. The IgG anti-klebsiella response was higher in B27-positive

Correspondence to Dr A Kijlstra, Netherlands Ophthalmic Research Institute, P O Box 12141, 1100 AC Amsterdam, The Netherlands.

patients without ankylosing spondylitis than in patients with ankylosing spondylitis.

Materials and methods

A total of 62 patients with anterior uveitis from the Ophthalmology Departments of the Erasmus University of Rotterdam, University of Amsterdam, and Free University of Amsterdam were included in this study. Serum was obtained from all patients during while the disease was active and from 33 healthy members of our institute.

All patients were seen by a rheumatologist. Physical examination included measuring the flexion capability of the lumbar spine by the modified technique of Schober (normal shift >5 cm) and examination of spinal movement in three planes. Chest expansion was determined at nipple level in males and below the breasts in females (normal shift >5 cm). X-rays of the sacroiliac joints were taken in anteroposterior view of the pelvis in a 25° craniocaudal direction. All radiographs were examined without the investigator's knowing which patient's radiograph he was observing. The diagnosis of ankylosing spondylitis was made according to the New York criteria.¹⁶

All patients were typed for the HLA-B27 antigen according to the National Institute of Health technique with four different commercially available HLA-B27 typing sera (Behring, Marburg, W Germany, one serum; Biotest, Frankfurt, W Germany, three sera. Patients and controls were subsequently divided in four groups consisting of healthy controls, B27-positive anterior uveitis patients with ankylosing spondylitis, B27-positive anterior uveitis patients without ankylosing spondylitis, and HLA-B27-negative anterior uveitis patients.

Total IgA and IgG serum levels were determined by radial immunodiffusion with precipitating rabbit antisera and a human standard serum containing 125 international units of IgG and 116 international units of IgA obtained from the Central Laboratory of Blood Transfusion (Amsterdam). IgA and IgG antibodies against klebsiella antigens were determined by an ELISA as described by Trull *et al.*¹⁵ with minor modifications.

The klebsiella K43 'cross-reactive' strain (obtained from Dr A F Geczy, Sydney, Australia) was grown in a Koser's citrate broth with 1% inositol¹⁷ overnight at 37°C. The protein content of the culture filtrate, obtained after centrifugation and passage through Millipore filters was measured by the Folin method using bovine serum albumin (BSA) as a standard. The filtrate was diluted to a protein concentration of 7.5 µg/ml in phosphate buffered saline, and micro-

cuvettes (Gilford, Cleveland, Ohio) were coated overnight at 37°C with 300 µl per cuvette. After being coated the cuvettes were washed three times with phosphate buffered saline containing 0.1% Tween (PBS 0.1% Tween). Subsequently 250 µl serum samples, diluted 1/25 in PBS 0.1% Tween containing 2 mg/ml BSA, were incubated for 2 hours at 37°C. After being washed three times with PBS 0.1% Tween the cuvettes were incubated with a 1/2000 dilution of heavy chain specific peroxidase conjugated goat anti-human IgA or IgG antibody respectively for 1 hour at 37°C (Nordic, Tilburg, The Netherlands). After being washed three times with PBS 0.1% Tween, bound peroxidase labelled antibody was developed at room temperature by adding 350 µl ABTS solution (0.16 mM 2,2'-azino-di-3-ethyl-benzthiazoline-6-sulphonate, Boehringer, Mannheim, W Germany; 0.15% H₂O₂ in 0.05 M citric acid, pH 4.0). The reaction was stopped by adding 50 µl 10% sodium dodecylsulphate to each cuvette. The green reaction product was measured in a spectrophotometer (EIA, Gilford, Cleveland, Ohio, USA) at 405nm. As a standard a serum sample was used from a pool of normal human sera. This standard was considered to contain 10 ELISA units of IgG and of IgA anti-klebsiella antibodies respectively.

Statistical analysis of the data was by Student's *t* test.

Results

The sex and age distributions of the various patient groups and controls are shown in Table 1. Serum IgA was significantly increased in the HLA-B27-positive anterior uveitis patients also suffering from ankylosing spondylitis as compared with controls ($p < 0.001$). The IgA levels in patients with HLA-B27-positive anterior uveitis without ankylosing spondylitis was somewhat less but still significantly ($p < 0.025$) increased as compared with controls. Other comparisons between the groups investigated did not show significant differences.

The IgA response against klebsiella was not statistically different between the groups of patients tested and controls. When the relative amount of IgA anti-klebsiella antibodies was calculated (ratio of anti-klebsiella IgA divided by total IgA) for each patient, it was found that the HLA-B27-positive anterior uveitis patients with ankylosing spondylitis had a significantly ($p < 0.01$) decreased ratio (Table 1). The ratios in the other patient groups were not different from those of the controls.

Serum levels of IgG (Table 2) were not altered in the patient groups investigated when compared with controls. Further, no differences were observed in the IgG anti-klebsiella antibodies between the

Table 1 Total serum IgA and IgA and klebsiella in anterior uveitis*

	n	Female	Male	Mean age	Total IgA	Anti-klebsiella IgA	Ratio
Controls	33	19	14	34	109±59	9.5±7.5	0.103±0.086
B27 ⁺ AU ⁺ AS ⁺	19	8	11	33	181±76†	7.2±3.7	0.050±0.037§
B27 ⁺ AU ⁺ AS ⁻	17	7	10	33	154±68‡	9.0±6.0	0.073±0.069
B27 ⁻ AU ⁺ AS ⁻	25	16	9	42	119±51	8.9±5.8	0.086±0.055

*Total IgA levels are expressed as international units, whereas anti-klebsiella IgA is expressed in local ELISA units. Data represent the mean ± standard deviation. Ratios represent anti-klebsiella IgA divided by total IgA as calculated for each individual.

†p<0.001 (compared with controls).

‡p<0.025 (compared with controls).

§p<0.01 (compared with controls).

patient groups and controls. The IgG anti-klebsiella response was, however, significantly higher in the B27⁺ AU⁺ AS⁻ group as compared with the B27⁺ AU⁺ AS⁺ group (p<0.01). Calculation of the ratio between klebsiella IgG antibodies and total serum IgG showed no differences between the patient groups and controls (Table 2). A comparison of these IgG ratios between the patient groups showed that the B27⁺ AU⁺ AS⁺ patient group had relatively less IgG anti-klebsiella antibodies compared with the B27⁺ AU⁺ AS⁻ group.

Discussion

This study shows that the total serum IgA level is increased in patients with HLA-B27-positive anterior uveitis. Similar findings were recently reported by McCoy *et al.*¹⁸ These observations indicate that antigenic mucosal stimulation may play a part in the pathogenesis of HLA-B27-associated anterior uveitis. Within this patient group those patients who also had ankylosing spondylitis had higher IgA levels than those without. Raised total IgA levels in ankylosing spondylitis have been reported elsewhere and were shown to be correlated with disease activity.¹⁴ In ankylosing spondylitis it has also been shown that IgA antibodies against klebsiella were increased in patients with active disease.¹⁵ Using a similar technique, we were unable

to find an alteration in the IgA response against a 'cross-reactive' *Klebsiella pneumoniae* strain in HLA-B27-positive uveitis patients. These findings indicate that the alleged stimulatory effect on the IgA synthesis seen in B27-positive uveitis patients does not result in a parallel increase in the IgA anti-klebsiella antibodies.

Several explanations are possible for these observations. First, it can be supposed that klebsiella organisms are not involved in the course of the disease at all, but that other mucosal inflammatory disorders, infectious or non-infectious, are the cause of the raised IgA levels in these patients. On the other hand it could be argued that only one klebsiella serotype was investigated in the study described here and that, furthermore, only the immune response against soluble bacterial antigens was tested. The strain used (obtained from Dr A F Geczy) has been found to contain antigens which may have an important role in HLA-B27-associated diseases.¹⁰ It has been shown to be a producer of 'modifying factor', a protein which is considered to be present on the lymphocyte membrane of most HLA-B27-positive ankylosing spondylitis patients and which was shown to interact with the lymphocytes of HLA-B27-positive healthy individuals. We may wonder whether any of this 'modifying factor' was present in the klebsiella culture filtrate used for coating the microcuvettes. Because for the ELISA technique the K43 strain was grown in a minimum essential medium, it is possible that certain bacterial antigens were not present in the culture filtrate, for it is known that the phenotypical expression of some bacterial antigens requires specific growth conditions.

A second possibility is that enteric infections with klebsiella organisms may be involved in the pathogenesis of the disease, but that this does not result in a stimulation of the klebsiella-specific immune response but rather in a polyclonal IgA stimulation.¹⁹ The hypothesis that a mucosal stimulation is involved is suggested by the fact that only the IgA levels but not the IgG levels are raised in the B27-positive uveitis patients.

Table 2 Total serum IgG and IgG anti-klebsiella in anterior uveitis*

		Anti-klebsiella IgG	Ratio
Controls	146±44	7.2±5.7	0.050±0.044
B27 ⁺ AU ⁺ AS ⁺	166±46	5.4±2.7	0.035±0.019
B27 ⁺ AU ⁺ AS ⁻	155±74	10.7±7.2†	0.079±0.054‡
B27 ⁻ AU ⁺ AS ⁻	143±43	8.1±6.0	0.066±0.065

*Total IgG levels are expressed as international units, whereas anti-klebsiella IgG is expressed in local ELISA units. Data represent the mean ± standard deviation. Ratios represent anti-klebsiella IgG divided by total IgG as calculated for each individual.

†p<0.001 (B27⁺ AU⁺ AS⁺ vs. B27⁺ AU⁺ AS⁻).

‡p<0.005 (B27⁺ AU⁺ AS⁺ vs. B27⁺ AU⁺ AS⁻).

Arguments which indicate that klebsiella organisms or cross-reactive bacteria do play a part in HLA-B27-associated uveitis stem from our finding that IgG levels against the klebsiella antigens were twice as high in the patients without ankylosing spondylitis than in those with ankylosing spondylitis. In conclusion, the findings reported here do not conclusively show an involvement of *Klebsiella pneumoniae* in the pathogenesis of HLA-B27-associated anterior uveitis, but they do suggest that factors resulting in an increased serum IgA play a part in this disease.

This study was performed with financial support from the Stichting Blinden-Penning and the Netherlands Rheumatism Association. We thank the ophthalmologists Dr M R Dandrieu, Dr S T T Tjoa, Dr P J Kruit, Dr A Rothova, Dr B J Christiaans, and Dr G S Baarsma for referring their patients, and Mrs J C M Beentjes for typing the manuscript. We thank Professor T E W Feltkamp for critically reviewing the manuscript.

References

- 1 Ebringer R, Cawdell DR, Ebringer AC. *Klebsiella pneumoniae* and acute anterior uveitis in ankylosing spondylitis. *Br Med J* 1979; **1**: 383.
- 2 Ebringer R, Cooke D, Cawdell DR, Cowling P, Ebringer A. Ankylosing spondylitis and HLA-B27. *Rheumatol Rehabil* 1977; **16**: 190-6.
- 3 Keat A. Reiter's syndrome and reactive arthritis in perspective. *N Engl J Med* 1983; **309**: 1606-15.
- 4 White L, McCoy R, Tait B, Ebringer R. A search for Gram-negative enteric micro-organisms in acute anterior uveitis: association of klebsiella with recent onset of disease, HLA-B27 and B27 creg. *Br J Ophthalmol* 1984; **68**: 750-5.
- 5 Warren RE, Brewerton DA. Klebsiella, spondylitis, and uveitis. *Br Med J* 1979; **i**: 889.
- 6 Willshaw HE. Acute anterior uveitis and *Klebsiella aerogenes*: a causal relationship? *Br J Ophthalmol* 1981; **65**: 796-7.
- 7 Ebringer RW, Cawdell DR, Cowling P, Ebringer A. Sequential studies in ankylosing spondylitis. Association of *Klebsiella pneumoniae* with active disease. *Ann Rheum Dis* 1978; **37**: 146-51.
- 8 Warren RE, Brewerton DA. Faecal carriage of klebsiella by patients with ankylosing spondylitis and rheumatoid arthritis. *Ann Rheum Dis* 1980; **39**: 37-44.
- 9 Eastmond CJ, Willshaw HE, Burgess SEP, Shinebaum R, Cooke EM, Wright V. Frequency of faecal *Klebsiella aerogenes* in patients with AS and controls with respect to individual features of the disease. *Ann Rheum Dis* 1980; **39**: 118-23.
- 9a Ebringer A. The cross-tolerance hypothesis, HLA-B27 and ankylosing spondylitis. *Br J Rheumatol* 1983; **22**: 53-66.
- 10 Geczy AF, Alexander K, Bashir HV, Edmonds JP, Upfold L, Sullivan J. HLA-B27. Klebsiella and ankylosing spondylitis; biological and chemical studies. *Immunol Rev* 1983; **70**: 23-50.
- 11 Beaulieu AD, Rousseau F, Israël-Assayag E, Roy R. Klebsiella related antigens in ankylosing spondylitis. *J Rheumatol* 1983; **10**: 102-5.
- 12 Archer JR. Search for cross-reactivity between HLA-B27 and *Klebsiella pneumoniae*. *Ann Rheum Dis* 1981; **40**: 400-3.
- 13 Veys EM, van Laere M. Serum IgG, IgM, and IgA levels in ankylosing spondylitis. *Ann Rheum Dis* 1973; **32**: 493-6.
- 14 Cowling P, Ebringer R, Ebringer A. Association of inflammation with raised serum IgA in ankylosing spondylitis. *Ann Rheum Dis* 1980; **39**: 545-9.
- 15 Trull A, Ebringer A, Panayi G, Ebringer R, James DCO. HLA-B27 and the immune response to enterobacterial antigens in ankylosing spondylitis. *Clin Exp Immunol* 1984; **55**: 74-80.
- 16 Bennett PH, Burch TA. New York symposium on population studies in rheumatic diseases: new diagnostic criteria. *Bull Rheum Dis* 1967; **17**: 453-8.
- 17 van Kregten E, Westerdaal NAC, Willers JMN. New simple medium for selective recovery of *Klebsiella pneumoniae* and *Klebsiella oxytoca* from human faeces. *J Clin Microbiol* 1984; **20**: 936-41.
- 18 McCoy R, White L, Tait B, Ebringer R. Serum immunoglobulins in acute anterior uveitis. *Br J Ophthalmol* 1984; **68**: 807-10.
- 19 Gross WL, Rucks A. *Klebsiella pneumoniae* stimulate highly purified human blood B cells to mature into plaque forming cells without prior proliferation. *Clin Exp Immunol* 1983; **52**: 372-80.

Accepted for publication 24 May 1985.