EXTENDED REPORT

Infectious background of patients with a history of acute anterior uveitis

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Objective: To study the infectious background of patients with a history of acute anterior uveitis (AAU) and healthy control subjects.

Methods: Sixty four patients with previous AAU and 64 sex and age matched controls were studied. Serum antibodies to *Salmonellae*, *Yersinia*, *Klebsiella pneumoniae*, *Escherichia coli*, *Proteus mirabilis*, *Campylobacter jejuni*, and *Borrelia burgdorferi* were measured using enzyme linked immunosorbent assay (ELISA), and antibodies to *Chlamydia trachomatis* and *Chlamydia pneumoniae* by microimmunofluorescence test. Peripheral blood mononuclear cells (PBMCs), separated by density gradient centrifugation, were studied for *Salmonella* and *Yersinia* antigens by means of an immunofluorescence test, and for *C pneumoniae* DNA with a polymerase chain reaction (PCR).

Results: Neither prevalence nor levels of single microbial antibodies studied differed between the patients and control subjects, or between subgroups of patients created on the basis of clinical characteristics. In logistic regression analysis, the high number of recurrences (>10) of AAU was independently related to the presence of single or multiple bacterial antibodies ($p=0.04$). None of the PBMC samples of the patients were positive for *Yersinia* or *Salmonella* antigens. *C pneumoniae* PCR was positive in a patient who was negative for *C pneumoniae* antibodies.

Conclusion: Although neither the prevalence nor the levels of single microbial antibodies studied differed between the patients and the controls, current data suggest that the presence of single or multiple antibodies in patients with many recurrences of AAU compared with patients with none or few recurrences may be a sign of repeated infections, antigen persistence, or raised innate immune responsiveness.

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Acute anterior uveitis (AAU) is a member of the spondyloarthropathy (SpA) family, which includes various seronegative arthritides such as ankylosing spondylitis (AS), a prototype of SpA, and reactive arthritis (ReA). Although the aetiopathology of SpA is not known, it is considered to involve genetic factors and environmental factors such as infections.¹ More than 50% of patients with AAU are positive for the HLA-B27 antigen.² AAU occurs in 5% of patients with acute ReA.³ Among patients with AAU but no signs of ReA, bacteria indicating an infectious cause are not often detected. If detected, they include gastrointestinal pathogens such as *Salmonellae* and *Yersinia*⁴⁻⁵ and urogenital pathogens such as *Chlamydia trachomatis*,⁶ all of which serve as triggers of ReA as well. *Borrelia burgdorferi* has also been associated with both AAU and ReA.⁷⁻⁸ Recently, *Chlamydia pneumoniae*, a respiratory tract pathogen, was associated with ReA⁹ and AAU.¹⁰ In patients with ReA, the presence of microbial antigens in the joint has been demonstrated for *Chlamydia trachomatis*,¹¹ *Yersinia*,¹² *Salmonella*,¹³ and *Shigella*.¹⁴ Also, microbial antigens can persist for prolonged periods in the circulation,¹⁵ in the gut, or in the skin.¹⁶ In AS, it has been suggested that *Klebsiella* species play a part in the exacerbation of the disease¹⁷ as well as in the development of AAU.¹⁸

Many aetiopathological features of AAU are shared with ReA. Unlike patients with ReA, little is known about antigen persistence in patients with AAU. We therefore measured the prevalence and serum levels of bacterial antibodies in patients with a history of AAU, and studied the presence of *Salmonella* and *Yersinia* antigens and *C pneumoniae* DNA in peripheral blood mononuclear cells (PBMCs). We compared the findings with healthy control subject material studied simultaneously.

PATIENTS AND METHODS

A total of 64 patients with idiopathic acute or recurrent anterior uveitis were first examined in the Helsinki University Eye Hospital between 1993 and 1996 and then again between September and December 1999 at a follow up visit. A visual acuity test, tonometry, a slit lamp examination, and an evaluation of the fundus with a 90 diopter lens, or indirect ophthalmoscopy, and a three mirror lens when necessary, were performed. Data on age, sex, age at onset of first uveitis, number of attacks, complications, and systemic symptoms and disorders were collected on standard forms. A rheumatological survey including a detailed history of the occurrence of musculoskeletal, abdominal, and skin diseases and completed with sacroiliac radiographic examination was performed on the patients with low back pain or peripheral joint symptoms suggestive of AS or other forms of SpA. Patients fulfilling the criteria of the European Spondylarthropathy Study Group were diagnosed as having seronegative SpA¹⁹. Sixty four healthy sex and age matched subjects of the hospital and laboratory staff or their acquaintances served as controls. They did not have a history of AAU or SpA.

Blood samples and routine laboratory tests

Serum samples for antibody detection were stored at -20°C until tested simultaneously. The PBMCs were isolated using

Abbreviations: AAU, acute anterior uveitis; AS, ankylosing spondylitis; EIA, enzyme immunoassay; ELISA, enzyme linked immunosorbent assay; MIF, microimmunofluorescence; PBMC, peripheral blood mononuclear cells; PCR, polymerase chain reaction; ReA, reactive arthritis; SpA, spondyloarthropathy

Vacutainer CPT cell preparation tubes with sodium citrate (Becton Dickinson Vacutainer Systems, Becton Dickinson and Co, Franklin Lakes, NJ, USA) according to the manufacturer's recommendations. From a proportion of the PBMC fraction, cytocentrifuge preparations were made and stored at -20°C until stained for microbial antigens. From another proportion, DNA was extracted using QIAamp DNAkit (Qiagen, Valencia, CA, USA) and stored at -70°C until used for *C pneumoniae* polymerase chain reaction (PCR). Routine laboratory tests (erythrocyte sedimentation rate, C reactive protein, blood cell count, *Treponema pallidum* haemagglutination assay, urine sediment) were also performed.

Antibodies to *Salmonellae*, *Yersiniae*, *Klebsiella pneumoniae*, *Escherichia coli*, and *Proteus mirabilis*

Yersinia enterocolitica O:3 and O:9, *Yersinia pseudotuberculosis* I and III, *E coli* and *P mirabilis* were clinical isolates. For *Salmonella*, combined lipopolysaccharides from *Salmonella enteritidis* and *Salmonella typhimurium* (Sigma Chemical Co, St. Louis, MO, USA) were used as antigens.²⁰ *S enteritidis*, *S typhimurium* and other *Salmonellae* belonging to groups B or D are responsible for about 90% of *Salmonella* infections diagnosed by bacterial isolation in Finland. The remaining *Salmonella* subtypes are probably also recognised by this ELISA, although with less efficiency.²⁰ *Klebsiella pneumoniae* strains 21 and 43 (Dr AF Geczy, New South Wales Red Cross Blood Transfusion Service, Sydney, Australia) and ATCC 27736 (the American Type Culture Collection, Rockville, MD, USA) were chosen on the basis of previous studies.^{21–23} The antigen extracts were prepared as previously described.²⁴

Polystyrene microtitre plates (Nunc, Roskilde, Denmark) were coated with the antigen preparations and then used to determine the prevalence of specific antibodies, as described previously.^{20–24} Antibody concentrations are expressed as enzyme immunoassay (EIA) units where one EIA unit is 1/100 of the corresponding antibody concentration in the positive reference serum. Antibody titres at least two standard deviations above the mean of a healthy control person were regarded as positive.

Antibodies to *Campylobacter jejuni*

The antigen for the ELISA measurement of antibodies to *Campylobacter* was an acid extract of *C jejuni* strain 143483 prepared and used as earlier described.²⁵ Titre levels of 3500 or more for IgG, 5000 for IgA, and 2500 for IgM were considered to indicate previous infection due to *C jejuni*.

Antibodies to *C pneumoniae* and *C trachomatis*

Antibodies specific for *C pneumoniae* and *C trachomatis* were both measured by the microimmunofluorescence (MIF)^{26,27} test. Antibody titres of $\geq 1/512$ in the IgG fraction, $\geq 1/16$ in the IgM fraction, or $\geq 1/160$ in the IgA fraction were considered to indicate ongoing infection, and a titre of 1/32–1/256 for IgG was considered to indicate previous infection. *C trachomatis* antibodies were also studied by MIF.²⁷

Antibodies to *B burgdorferi*

IgG and IgM antibodies to *B burgdorferi* were measured using the Lyme ELISA (Dako, Glostrup, Denmark), modified by end point titration.²⁸

Immunofluorescence staining of peripheral blood mononuclear cells for *Salmonella* and *Yersinia* antigens

The cytocentrifuge slides were stained with mouse monoclonal antibodies specific for O-polysaccharide chains of lipopolysaccharides of *S enteritidis* (MASE O9),²⁹ *S typhimurium* (MAST O4),²⁹ *Y enterocolitica* O:3 (A6),^{12,15,30} and the heat shock protein of *Y enterocolitica* O:3 (IV7D2).^{15,31} Each slide was read by one of the authors (KG)—who was blinded to the source of the cells—and were analysed using a fluorescence microscope

(Leitz diaphan incidence light fluorescence microscope with an Osram HBO 100 watt mercury lamp (Leitz, Wetzlar, Germany).

C pneumoniae polymerase chain reaction

C pneumoniae DNA was detected as described previously.^{32,33} The DNA preparation at -70°C was thawed and amplified by *C pneumoniae* specific nested primers in a touchdown PCR. The amplified products were detected by agarose gel electrophoresis.

HLA-B27 typing

The presence of HLA-B27 antigen was determined using either the standard microlymphocytotoxicity technique or PCR of the diagnostic molecular genetics laboratory of the hospital. Briefly, DNA was amplified with primers B27/E136 as 5'-CGG CGG TCC AGG AGC T-3' 5'- and B27/E91 s + 5'-GGG TCT CAC ACC CTC CAG AAT-3'. Generation of the HLA-B27 PCR product was detected on agarose gel electrophoresis.

Compliance with research ethics standards

The study was approved by the local ethics committee and conducted according to the tenets of the Declaration of Helsinki. Informed consent was obtained from all the patients and control subjects.

Statistical analysis

The variables had a non-normal distribution and were analysed with the Mann-Whitney U test. Categorical data were analysed by Pearson's χ^2 test or Fisher's exact test. To estimate dependencies between the presence of antibodies and the patients' characteristics we used a logistic regression model. Clinically important variables were chosen for the analysis. The forward selection method was used and cut off values were set at 0.2 for the variable selection.

RESULTS

Patients

There were 64 patients (38 men and 26 women) with a mean age of 45.4 (SD 12.8) years at the follow up. Fifty five (86%) of the patients and six (9%) of the sex and age matched healthy control subjects were HLA-B27 positive. Eighty four per cent of the patients had had a recurrent attack. The mean age at the onset of the first AAU was 34.2 (SD 11.6, range 14–64) years, and time between the first attack and the follow up visit was 11.1 (SD 9.6) years.

Twenty five per cent of the patients had developed SpA; 27% had been affected by eye complications (persistent synchia, cataract, cystoid macular degeneration, and posterior or panuveitis). Seven patients had more than one complication in each eye, and three patients had complications in both eyes. In 11% of the patients anterior uveitis had become chronic. The ocular complications were not associated with the presence of SpA (table 1).

Occurrence of antibodies in patients and control subjects

The prevalence of the antibodies to *Y enterocolitica* and *Y pseudotuberculosis* III, *Salmonellae*, *K pneumoniae*, *P mirabilis*, *E coli*, *Chlamydia*, and *Borrelia* species for the patients and the control subjects was much the same (table 2). Patients with AAU had antibodies to *Y pseudotuberculosis* I more often than the control subjects (28% v 11%, $p=0.01$; table 2). However, if corrected for the number of comparisons performed, the difference was not significant.

The antibody levels against enterobacteriaceae (*Yersiniae*, *K pneumoniae*, *C jejuni*, *E coli*, and *P mirabilis*), *C pneumoniae*, *C trachomatis*, and *B burgdorferi*, did not differ between the patients and the control subjects. An exception was the higher level of IgG antibodies to *Salmonellae* found in the controls ($p=0.018$).

Table 1 Characteristics of the 64 patients with previous acute anterior uveitis (AAU) at the time of the follow-up examination

Characteristics	Patients n	%
HLA-B27 antigen positivity	55	86
Recurrences of AAU:		
None	10	16
≤10	32	50
>10	22	34
Chronic eye disease*	7	11
Eye complications	17	27
Cataract†	9	7
Cystoid macular degeneration†	5	4
Glaucoma†	0	0
Persistent synechiae†	13	10
Posterior uveitis or panuveitis†	2	2
More than one complication per eye	7	11
Complications in both eyes	3	5
Spondyloarthropathy	16	25

*Iritis not resolved within three months; †data are number of eyes affected with complications.

Increased IgM antibodies to *Y pseudotuberculosis* I were measured more often in the patients than controls ($p=0.03$). When corrected for the number of comparisons made, the results were not significant.

Occurrence of antibodies in subgroups of patients

Sex, age at examination, eye complications, development of SpA, recurrences, time since the first AAU, HLA-B27 antigen, or chronic course of the disease did not correlate with antibody positivity (table 3). When a detailed analysis was made, the patients with eye complications had higher levels of IgA antibodies to *P mirabilis* ($p=0.003$ when corrected for the numbers of comparisons, $p=0.042$) than those without eye complications.

Four male patients and one female patient had a recurrent uveitis attack at the time of the examination. None of the patients had symptoms of clinical infection within six months before the recruitment or antibody levels diagnostic of recent infection. In the logistic regression analysis to explore whether a dependency between positive antibody levels (≥ 4 standard deviations of controls for *Enterobacteriaceae* and cut off values as mentioned in methods section for *Campylobacter*, *Borrelia*, and *Chlamydia* species) and the patients' characteristics existed, the independent variables were sex, age at onset of AAU, ocular complications, spondyloarthropathies, time since the first AAU, and number of recurrences (≤ 10 recurrences $v > 10$ recurrences). The number of the recurrences was the only variable of significance in relation to presence of antibodies against one or several bacteria ($p=0.047$).

Antigen persistence

None of the patients had persisting *Yersinia* or *Salmonella* antigens in their PBMCs. *C pneumoniae* DNA was detected in one of the patients who was not positive for *C pneumoniae* antibodies. All the controls were negative for *C pneumoniae* DNA.

DISCUSSION

In our study, patients with a history of many recurrences of AAU had raised antimicrobial antibody levels against one or several bacteria tested more often than patients with none or few recurrences. Such a high prevalence of antibodies could be a mark of subclinical infections in the gut or in the urogenital region or of increased penetrance of microbial antigens. Ebringer *et al* were the first to show that isolation of *Klebsiella* from faecal samples is related to the activation of AS and uveitis.³⁴ Later, it was found that antibodies occur against different intestinal and urogenital microbes in association with

Table 2 Prevalence of antibodies in patients (n=64) and control subjects (n=64)

Prevalence of antibodies	Patients n (%)	Controls n (%)
<i>Salmonellae</i> :		
IgA	4 (6)	5 (8)
IgG	2 (3)	2 (3)
IgM	5 (8)	3 (5)
Total positive	10 (16)	8 (13)
<i>Yersinia enterocolitica</i> 0:3:		
IgA	2 (3)	2 (3)
IgG	3 (5)	3 (5)
IgM	1 (2)	5 (8)
Total positive	6 (9)	9 (14)
<i>Yersinia enterocolitica</i> 0:9:		
IgA	0 (0)	2 (3)
IgG	2 (3)	4 (6)
IgM	3 (5)	4 (6)
Total positive	5 (8)	8 (13)
<i>Yersinia pseudotuberculosis</i> I:		
IgA	3 (5)	3 (5)
IgG	6 (9)	3 (5)
IgM	10 (16)	2 (3)
Total positive	18 (28)*	7 (11)
<i>Yersinia pseudotuberculosis</i> III:		
IgA	4 (6)	4 (6)
IgG	1 (2)	4 (6)
IgM	4 (6)	3 (5)
Total positive	9 (14)	9 (14)
<i>Klebsiella pneumoniae</i> strain 43:		
IgA	10 (16)	3 (5)
IgG	3 (5)	4 (6)
IgM	5 (8)	4 (6)
Total positive	14 (22)	9 (14)
<i>Klebsiella pneumoniae</i> strain 21:		
IgA	2 (3)	3 (5)
IgG	3 (5)	3 (5)
IgM	2 (3)	3 (5)
Total positive	5 (8)	8 (13)
<i>Klebsiella pneumoniae</i> strain ATCC27736:		
IgA	6 (9)	4 (6)
IgG	1 (2)	3 (5)
IgM	1 (2)	2 (3)
Total positive	7 (11)	7 (11)
<i>Campylobacter jejuni</i> :		
IgA	0 (0)	0 (0)
IgG	4 (6)	2 (3)
IgM	0 (0)	0 (0)
Total positive	4 (6)	2 (3)
<i>Proteus mirabilis</i> :		
IgA	5 (8)	3 (5)
IgG	1 (2)	3 (5)
IgM	5 (8)	3 (5)
Total positive	9 (14)	8 (13)
<i>Escherichia coli</i> :		
IgA	3 (5)	5 (8)
IgG	5 (8)	2 (3)
IgM	2 (3)	6 (9)
Total positive	6 (9)	13 (20)
<i>Chlamydia trachomatis</i> :		
IgA	1 (2)	0 (0)
IgG	3 (5)	4 (6)
IgM	0 (0)	0 (0)
Total positive	3 (5)	4 (6)
<i>Chlamydia pneumoniae</i> :		
IgA	2 (3)	22 (34)
IgG	36 (56)	46 (72)
IgM	2 (3)	0 (0)
Total positive	38 (59)	46 (72)
<i>Borrelia burgdorferi</i> :		
IgG	0 (0)	1 (2)
IgM	0 (0)	1 (2)
Total positive	0 (0)	2 (3)

*Pearson's χ^2 test, $p=0.01$.

Table 3 Presence of antibodies in relation to clinical characteristics of the patients

Characteristics	Patients without antibodies n	Patients with antibodies* n (%)
Sex:		
Men	5	33 (87)
Women	3	23 (88)
Age at examination:		
≤40 years	4	22 (85)
>40 years	4	34 (89)
Years from the first AAU:		
≤10 years	6	39 (87)
>10 years	2	17 (89)
Eye complications:		
None	4	43 (91)
Yes	4	13 (76)
Spondyloarthropathy:		
None	7	41 (85)
Yes	1	15 (94)
HLA-B27 antigen:		
Negative	2	7 (78)
Positive	6	49 (89)
Chronic eye disease:†		
No	7	50 (88)
Yes	1	6 (86)

*Table 2 presents the prevalence of antibodies to individual bacteria.
†Iritis not resolved within three months.

AAU in patients with AS, such as increased IgA antibody levels against *K pneumoniae* and *E coli* lipopolysaccharide³⁵ and higher frequency of IgG antibodies to *C trachomatis*.³⁶ We found in the present study that the patients with eye complications had increased levels of IgA antibodies against *P mirabilis*. The high prevalence of antibodies found may indicate that the patients had been exposed to the bacteria in the past. A single increased antibody level may derive from prolonged antigen persistence as found in patients with SpA¹⁶ and ReA.^{37,38} Bacterial antigens, DNA, and RNA have been shown to persist in the synovial membrane,³⁹⁻⁴² synovial fluid cells,^{11-14,43-45} and peripheral blood cells^{13,15,46,47} in patients with ReA. However, the pathogenetic implications of antigen persistence remain uncertain because *Chlamydia* DNA has also been found in the joints of patients with rheumatoid arthritis,⁴⁶ and in asymptomatic subjects,⁴⁷ and *Shigella* or *Yersinia* antigens have been found in peripheral blood cells in patients years after uncomplicated enteric infections.^{14,15} The negative findings in the search for antigenic material in PBMCs in our study do not support the hypothesis that antigen persistence would be a common feature in patients with previous AAU.

In experimental studies, bacterial invasion into HLA-B27 transfected cells has been shown to be decreased,⁴⁸ unchanged,⁴⁹ or increased.⁵⁰ Although rats and mice transgenic for HLA-B27 show enhanced susceptibility to bacterial infections,^{51,52} the expression of HLA-B27 does not influence the incidence or severity of uveitis in the bacterially induced AAU model.⁵³ HLA-B27 diminished elimination of *Salmonella* in monocytic U937 cells transfected with HLA-B27.⁵⁴

The raised antibody levels found in the present study may derive from high innate immune responsiveness. In accordance with this possibility, Ikawa *et al* used Hela cells transfected with HLA-B27 gene to provide evidence suggesting that HLA-B27 may be associated with the activation of otherwise silent intracellular signal transduction pathways and may also contribute to the activation of innate immune genes.⁵⁵ In addition, Ekman has shown that the impaired capacity of *Salmonella* permissive, HLA-B27 transfected, human monocytic U937 cells to clear *Salmonella* is associated with the high production of tumour necrosis factor α and interleukin 10.⁵⁶ These findings, obtained under in vitro conditions, are indeed in agreement with our recently reported results showing raised tumour necrosis factor α pro-

duction in patients with previous AAU compared with healthy controls.⁵⁷ Taken together, the mechanisms described above may enhance phagocytic antigen processing or presentation capacity at the early steps of an immune response.⁵⁸

To conclude, current data suggest that raised antibody responses in this group of patients with a high frequency of HLA-B27 and with many recurrences of AAU compared with patients with none or few recurrences may be a sign of repeated infections, antigen persistence, or innate immune responsiveness.

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